

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
26 June 2003 (26.06.2003)

PCT

(10) International Publication Number  
**WO 03/052068 A2**

(51) International Patent Classification<sup>7</sup>: **C12N**  
(21) International Application Number: PCT/US02/39796  
(22) International Filing Date:  
12 December 2002 (12.12.2002)  
(25) Filing Language: English  
(26) Publication Language: English  
(30) Priority Data:  
60/340,213 13 December 2001 (13.12.2001) US  
60/340,314 13 December 2001 (13.12.2001) US  
60/340,322 13 December 2001 (13.12.2001) US  
60/357,502 15 February 2002 (15.02.2002) US

(71) Applicant (*for all designated States except US*): **EX-ELIXIS, INC.** [US/US]; P.O. Box 511, 170 Harbor Way, South San Francisco, CA 94083-0511 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **COSTA, Michael, A.** [US/US]; 18 Hazelwood Avenue, San Francisco, CA 94112 (US). **GENDREAU, Steven, Brian** [US/US]; 2801 Turk Street, #3, San Francisco, CA 94118 (US). **DORA, Emery, G., III** [US/US]; 1847 32nd Avenue, San Francisco, CA 94122 (US). **NICOLL, Monique** [US/US]; 224 Naomi Avenue, Pacifica, CA 94044 (US).

(74) Agents: **SHAYESTEH, Laleh et al.**; Exelixis, Inc., P.O. Box 511, 170 Harbor Way, South San Francisco, CA 94083-0511 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— *without international search report and to be republished upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: **MBCATS AS MODIFIERS OF THE BETA-CATENIN PATHWAY AND METHODS OF USE**

(57) Abstract: Human MBCAT genes are identified as modulators of the beta-catenin pathway, and thus are therapeutic targets for disorders associated with defective beta-catenin function. Methods for identifying modulators of beta-catenin, comprising screening for agents that modulate the activity of MBCAT are provided.

## MBCATs AS MODIFIERS OF THE BETA-CATENIN PATHWAY AND METHODS OF USE

### REFERENCE TO RELATED APPLICATIONS

5           This application claims priority to U.S. provisional patent applications 60/340,213 filed 12/13/2001, 60/340,314, filed 12/13/2001, 60/340,322 filed 12/13/2001, and 60/357,502 filed 2/15/2002. The contents of the prior applications are hereby incorporated in their entirety.

### 10                           BACKGROUND OF THE INVENTION

          Beta-catenin is an adherens junction protein. Adherens junctions (AJs; also called the zonula adherens) are critical for the establishment and maintenance of epithelial layers, such as those lining organ surfaces. AJs mediate adhesion between cells, communicate a signal that neighboring cells are present, and anchor the actin cytoskeleton. In serving  
15   these roles, AJs regulate normal cell growth and behavior. At several stages of embryogenesis, wound healing, and tumor cell metastasis, cells form and leave epithelia. This process, which involves the disruption and reestablishment of epithelial cell-cell contacts, may be regulated by the disassembly and assembly of AJs. AJs may also function in the transmission of the 'contact inhibition' signal, which instructs cells to stop  
20   dividing once an epithelial sheet is complete.

          The AJ is a multiprotein complex assembled around calcium-regulated cell adhesion molecules called cadherins (Peifer, M.(1993) Science 262: 1667-1668). Cadherins are transmembrane proteins: the extracellular domain mediates homotypic adhesion with cadherins on neighboring cells, and the intracellular domain interacts with  
25   cytoplasmic proteins that transmit the adhesion signal and anchor the AJ to the actin cytoskeleton. These cytoplasmic proteins include the alpha-, beta-, and gamma-catenins. The beta-catenin protein shares 70% amino acid identity with both plakoglobin, which is found in desmosomes (another type of intracellular junction), and the product of the Drosophila segment polarity gene 'armadillo'. Armadillo is part of a multiprotein AJ  
30   complex in Drosophila that also includes some homologs of alpha-catenin and cadherin, and genetic studies indicate that it is required for cell adhesion and cytoskeletal integrity.

          Beta-catenin, in addition to its role as a cell adhesion component, also functions as a transcriptional co-activator in the Wnt signaling pathway through its interactions with

the family of Tcf and Lef transcription factors (for a review see Polakis, (1999) Current Opinion in Genetics & Development, 9:15-21 and Gat U., et al., (1998) Cell 95:605-614).

The APC gene, which is mutant in adenomatous polyposis of the colon, is a negative regulator of beta-catenin signaling (Korinek, V. et al., (1997) Science 275: 1784-1787; Morin, P. J., et al., (1997) Science 275: 1787-1790). The APC protein normally binds to beta-catenin and, in combination with other proteins (including glycogen synthase kinase-3b and axin, is required for the efficient degradation of b-catenin. The regulation of beta-catenin is critical to the tumor suppressive effect of APC and that this regulation can be circumvented by mutations in either APC or beta-catenin.

While mammals contain only a single beta-catenin gene, *C. elegans* contains three (Korswagen HC, et al., (2000) Nature 406:527-32). Each worm beta-catenin appears to carry out unique functions (Korswagen HC, et al., (2000) Nature 406:527-32, Nartarajan L et al. (2001) Genetics 159: 159-72). Because of the divergence of function in *C. elegans*, it is possible to specifically study beta-catenin role in cell adhesion, which is mediated by the *C. elegans* beta-catenin HMP-2.

The *Drosophila* 'discs large' tumor suppressor protein, Dlg, is the prototype of a family of proteins termed MAGUKs (membrane-associated guanylate kinase homologs). MAGUKs are localized at the membrane-cytoskeleton interface, usually at cell-cell junctions, where they appear to have both structural and signaling roles. They contain several distinct domains, including a modified guanylate kinase domain, an SH3 motif, and 1 or 3 copies of the DHR (GLGF/PDZ) domain. Recessive lethal mutations in the 'discs large' tumor suppressor gene interfere with the formation of septate junctions (thought to be the arthropod equivalent of tight junctions) between epithelial cells, and they also cause neoplastic overgrowth of imaginal discs, suggesting a role for cell junctions in proliferation control. A homolog of the *Drosophila* Dlg protein was isolated from human B lymphocytes (Lue et al., (1994) Proc. Nat. Acad. Sci. 91: 9818-9822) and shown to bind directly to the membrane cytoskeletal protein 4.1. The presence of human DLG isoforms with or without the protein 4.1 binding domain suggested that the tissue-specific cytoskeletal interactions of the protein may be regulated by alternative splicing of its transcripts.

DLG1 may function as a coupler of tyrosine kinase and a voltage-gated potassium channel in T lymphocytes (Hanada, T., et al., (1997) J. Biol. Chem. 272: 26899-26904). In *Drosophila*, lethal giant larvae (Lgl) is essential for asymmetric cortical localization of all basal determinants in mitotic neuroblasts, and is therefore indispensable for neural fate

decisions (Ohshiro, T., et al., (2000) Nature 408: 593-596). Lgl, which itself is uniformly cortical, interacts with several types of myosin to localize the determinants. Dlg, another tumor suppressor gene, participates in this process by regulating the localization of Lgl. The localization of the apical components is unaffected in Lgl or Dlg mutants. Thus, Lgl and Dlg act in a common process that differentially mediates cortical protein targeting in mitotic neuroblasts, and creates intrinsic differences between daughter cells.

DLG2, DLG3, and DLG4 are also members of the MAGUK family.

DLG2 (chapsyn-110) and DLG4 (PSD95) each mediate the clustering of NMDA receptors and potassium channels. The proper distribution of voltage-gated and ligand-gated ion channels on the neuronal surface is critical for the processing and transmission of electrical signals in neurons. Chapsyn-110 and PSD95 heteromultimerize with each other and are recruited into the same NMDA receptor and potassium channel clusters. These 2 MAGUK proteins may interact at postsynaptic sites to form a multimeric scaffold for the clustering of receptors, ion channels, and associated signaling proteins (Kim, E., et al., (1996) Neuron 17: 103-113).

DLG3 interacts with the C-terminal region of the APC tumor suppressor protein, and may negatively regulate cell proliferation through its interaction with the APC protein (Makino, K., et al., (1997) Oncogene 14: 2425-2433).

Organ specific differentiation is effected through cell-cell interactions. Mesenchymal stromal cells act to direct growth and differentiation of epithelial cells. Such stromal-epithelial interactions lead to complete organogenesis and maintenance of homeostasis in adult tissues. In cancer, carcinoma epithelial cells induce a reactive stroma. Reactive stroma differentially influences rate of tumorigenesis through unknown mechanisms. Defining specific mechanisms of reciprocal stromal-epithelial signaling and genes and proteins involved in the system, phenotypic switching of stroma, and the role of reactive stroma in cancer progression is therefore highly desired.

The ability to manipulate the genomes of model organisms such as *C. elegans* provides a powerful means to analyze biochemical processes that, due to significant evolutionary conservation, have direct relevance to more complex vertebrate organisms. Due to a high level of gene and pathway conservation, the strong similarity of cellular processes, and the functional conservation of genes between these model organisms and mammals, identification of the involvement of novel genes in particular pathways and their functions in such model organisms can directly contribute to the understanding of the correlative pathways and methods of modulating them in mammals (see, for example,

Dulubova I, et al, J Neurochem 2001 Apr;77(1):229-38; Cai T, et al., Diabetologia 2001 Jan;44(1):81-8; Pasquinelli AE, et al., Nature. 2000 Nov 2;408(6808):37-8; Ivanov IP, et al., EMBO J 2000 Apr 17;19(8):1907-17; Vajo Z et al., Mamm Genome 1999 Oct;10(10):1000-4). For example, a genetic screen can be carried out in an invertebrate

5 model organism having underexpression (e.g. knockout) or overexpression of a gene (referred to as a "genetic entry point") that yields a visible phenotype. Additional genes are mutated in a random or targeted manner. When a gene mutation changes the original phenotype caused by the mutation in the genetic entry point, the gene is identified as a "modifier" involved in the same or overlapping pathway as the genetic entry point. When  
10 the genetic entry point is an ortholog of a human gene implicated in a disease pathway, such as beta-catenin, modifier genes can be identified that may be attractive candidate targets for novel therapeutics.

All references cited herein, including patents, patent applications, publications, and sequence information in referenced Genbank identifier numbers, are incorporated herein in  
15 their entireties.

### SUMMARY OF THE INVENTION

We have discovered genes that modify the beta-catenin pathway in *C. elegans*, and identified their human orthologs, hereinafter referred to as modifiers of beta-catenin  
20 (MBCAT). The invention provides methods for utilizing these beta-catenin modifier genes and polypeptides to identify MBCAT-modulating agents that are candidate therapeutic agents that can be used in the treatment of disorders associated with defective or impaired beta-catenin function and/or MBCAT function. Preferred MBCAT-modulating agents specifically bind to MBCAT polypeptides and restore beta-catenin  
25 function. Other preferred MBCAT-modulating agents are nucleic acid modulators such as antisense oligomers and RNAi that repress MBCAT gene expression or product activity by, for example, binding to and inhibiting the respective nucleic acid (i.e. DNA or mRNA).

MBCAT modulating agents may be evaluated by any convenient *in vitro* or *in vivo*  
30 assay for molecular interaction with an MBCAT polypeptide or nucleic acid. In one embodiment, candidate MBCAT modulating agents are tested with an assay system comprising a MBCAT polypeptide or nucleic acid. Agents that produce a change in the activity of the assay system relative to controls are identified as candidate beta-catenin modulating agents. The assay system may be cell-based or cell-free. MBCAT-

modulating agents include MBCAT related proteins (e.g. dominant negative mutants, and biotherapeutics); MBCAT-specific antibodies; MBCAT-specific antisense oligomers and other nucleic acid modulators; and chemical agents that specifically bind to or interact with MBCAT or compete with MBCAT binding partner (e.g. by binding to an MBCAT binding partner). In one specific embodiment, a small molecule modulator is identified using a binding assay. In specific embodiments, the screening assay system is selected from an apoptosis assay, a cell proliferation assay, an angiogenesis assay, and a hypoxic induction assay.

In another embodiment, candidate beta-catenin pathway modulating agents are further tested using a second assay system that detects changes in the beta-catenin pathway, such as angiogenic, apoptotic, or cell proliferation changes produced by the originally identified candidate agent or an agent derived from the original agent. The second assay system may use cultured cells or non-human animals. In specific embodiments, the secondary assay system uses non-human animals, including animals predetermined to have a disease or disorder implicating the beta-catenin pathway, such as an angiogenic, apoptotic, or cell proliferation disorder (e.g. cancer).

The invention further provides methods for modulating the MBCAT function and/or the beta-catenin pathway in a mammalian cell by contacting the mammalian cell with an agent that specifically binds a MBCAT polypeptide or nucleic acid. The agent may be a small molecule modulator, a nucleic acid modulator, or an antibody and may be administered to a mammalian animal predetermined to have a pathology associated the beta-catenin pathway.

### DETAILED DESCRIPTION OF THE INVENTION

Genetic screens were designed to identify modifiers of the beta-catenin pathway in *C. elegans*. A weak allele of beta-catenin was used in our screen (a homozygous viable mutant of beta-catenin, allele qm39). The hmp-2 (qm-39) strain produces larval worms with a highly penetrant lumpy body phenotype in first stage larval worms (L1s). Various specific genes were silenced by RNA inhibition (RNAi). Methods for using RNAi to silence genes in *C. elegans* are known in the art (Fire A, et al., 1998 Nature 391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); WO9932619). Genes causing altered phenotypes in the worms were identified as modifiers of the beta-catenin pathway. Accordingly, vertebrate orthologs of these modifiers, and preferably the human orthologs, MBCAT genes (i.e., nucleic acids and polypeptides) are attractive drug targets for the

treatment of pathologies associated with a defective beta-catenin signaling pathway, such as cancer. Table 1 (Example II) lists the modifiers and their orthologs.

In vitro and in vivo methods of assessing MBCAT function are provided herein. Modulation of the MBCAT or their respective binding partners is useful for understanding the association of the beta-catenin pathway and its members in normal and disease conditions and for developing diagnostics and therapeutic modalities for beta-catenin related pathologies. MBCAT-modulating agents that act by inhibiting or enhancing MBCAT expression, directly or indirectly, for example, by affecting an MBCAT function such as enzymatic (e.g., catalytic) or binding activity, can be identified using methods provided herein. MBCAT modulating agents are useful in diagnosis, therapy and pharmaceutical development.

### **Nucleic acids and polypeptides of the invention**

Sequences related to MBCAT nucleic acids and polypeptides that can be used in the invention are disclosed in Genbank (referenced by Genbank identifier (GI) or RefSeq number), and shown in Table 1.

The term "MBCAT polypeptide" refers to a full-length MBCAT protein or a functionally active fragment or derivative thereof. A "functionally active" MBCAT fragment or derivative exhibits one or more functional activities associated with a full-length, wild-type MBCAT protein, such as antigenic or immunogenic activity, enzymatic activity, ability to bind natural cellular substrates, etc. The functional activity of MBCAT proteins, derivatives and fragments can be assayed by various methods known to one skilled in the art (Current Protocols in Protein Science (1998) Coligan *et al.*, eds., John Wiley & Sons, Inc., Somerset, New Jersey) and as further discussed below. In one embodiment, a functionally active MBCAT polypeptide is a MBCAT derivative capable of rescuing defective endogenous MBCAT activity, such as in cell based or animal assays; the rescuing derivative may be from the same or a different species. For purposes herein, functionally active fragments also include those fragments that comprise one or more structural domains of an MBCAT, such as a kinase domain or a binding domain. Protein domains can be identified using the PFAM program (Bateman A., et al., Nucleic Acids Res, 1999, 27:260-2). Methods for obtaining MBCAT polypeptides are also further described below. In some embodiments, preferred fragments are functionally active, domain-containing fragments comprising at least 25 contiguous amino acids, preferably at least 50, more preferably 75, and most preferably at least 100 contiguous amino acids of

any one of SEQ ID NOs:10-16 (an MBCAT). In further preferred embodiments, the fragment comprises the entire functionally active domain.

The term "MBCAT nucleic acid" refers to a DNA or RNA molecule that encodes a MBCAT polypeptide. Preferably, the MBCAT polypeptide or nucleic acid or fragment thereof is from a human, but can also be an ortholog, or derivative thereof with at least 70% sequence identity, preferably at least 80%, more preferably 85%, still more preferably 90%, and most preferably at least 95% sequence identity with human MBCAT. Methods of identifying orthologs are known in the art. Normally, orthologs in different species retain the same function, due to presence of one or more protein motifs and/or 3-dimensional structures. Orthologs are generally identified by sequence homology analysis, such as BLAST analysis, usually using protein bait sequences. Sequences are assigned as a potential ortholog if the best hit sequence from the forward BLAST result retrieves the original query sequence in the reverse BLAST (Huynen MA and Bork P, Proc Natl Acad Sci (1998) 95:5849-5856; Huynen MA *et al.*, Genome Research (2000) 10:1204-1210). Programs for multiple sequence alignment, such as CLUSTAL (Thompson JD *et al.*, 1994, Nucleic Acids Res 22:4673-4680) may be used to highlight conserved regions and/or residues of orthologous proteins and to generate phylogenetic trees. In a phylogenetic tree representing multiple homologous sequences from diverse species (e.g., retrieved through BLAST analysis), orthologous sequences from two species generally appear closest on the tree with respect to all other sequences from these two species. Structural threading or other analysis of protein folding (e.g., using software by ProCeryon, Biosciences, Salzburg, Austria) may also identify potential orthologs. In evolution, when a gene duplication event follows speciation, a single gene in one species, such as *C. elegans*, may correspond to multiple genes (paralogs) in another, such as human. As used herein, the term "orthologs" encompasses paralogs. As used herein, "percent (%) sequence identity" with respect to a subject sequence, or a specified portion of a subject sequence, is defined as the percentage of nucleotides or amino acids in the candidate derivative sequence identical with the nucleotides or amino acids in the subject sequence (or specified portion thereof), after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent sequence identity, as generated by the program WU-BLAST-2.0a19 (Altschul *et al.*, J. Mol. Biol. (1997) 215:403-410) with all the search parameters set to default values. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the



sequence of interest is being searched. A % identity value is determined by the number of matching identical nucleotides or amino acids divided by the sequence length for which the percent identity is being reported. "Percent (%) amino acid sequence similarity" is determined by doing the same calculation as for determining % amino acid sequence identity, but including conservative amino acid substitutions in addition to identical amino acids in the computation.

A conservative amino acid substitution is one in which an amino acid is substituted for another amino acid having similar properties such that the folding or activity of the protein is not significantly affected. Aromatic amino acids that can be substituted for each other are phenylalanine, tryptophan, and tyrosine; interchangeable hydrophobic amino acids are leucine, isoleucine, methionine, and valine; interchangeable polar amino acids are glutamine and asparagine; interchangeable basic amino acids are arginine, lysine and histidine; interchangeable acidic amino acids are aspartic acid and glutamic acid; and interchangeable small amino acids are alanine, serine, threonine, cysteine and glycine.

Alternatively, an alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman (Smith and Waterman, 1981, *Advances in Applied Mathematics* 2:482-489; database: European Bioinformatics Institute; Smith and Waterman, 1981, *J. of Molec.Biol.*, 147:195-197; Nicholas et al., 1998, "A Tutorial on Searching Sequence Databases and Sequence Scoring Methods" ([www.psc.edu](http://www.psc.edu)) and references cited therein.; W.R. Pearson, 1991, *Genomics* 11:635-650). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff (Dayhoff: *Atlas of Protein Sequences and Structure*, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA), and normalized by Gribskov (Gribskov 1986 *Nucl. Acids Res.* 14(6):6745-6763). The Smith-Waterman algorithm may be employed where default parameters are used for scoring (for example, gap open penalty of 12, gap extension penalty of two). From the data generated, the "Match" value reflects "sequence identity."

Derivative nucleic acid molecules of the subject nucleic acid molecules include sequences that hybridize to the nucleic acid sequence of any of SEQ ID NOs:1-9. The stringency of hybridization can be controlled by temperature, ionic strength, pH, and the presence of denaturing agents such as formamide during hybridization and washing. Conditions routinely used are set out in readily available procedure texts (*e.g.*, *Current Protocol in Molecular Biology*, Vol. 1, Chap. 2.10, John Wiley & Sons, Publishers (1994); Sambrook *et al.*, *Molecular Cloning*, Cold Spring Harbor (1989)). In some embodiments,

a nucleic acid molecule of the invention is capable of hybridizing to a nucleic acid molecule containing the nucleotide sequence of any one of SEQ ID NOs:1-9 under high stringency hybridization conditions that are: prehybridization of filters containing nucleic acid for 8 hours to overnight at 65° C in a solution comprising 6X single strength citrate (SSC) (1X SSC is 0.15 M NaCl, 0.015 M Na citrate; pH 7.0), 5X Denhardt's solution, 0.05% sodium pyrophosphate and 100 µg/ml herring sperm DNA; hybridization for 18-20 hours at 65° C in a solution containing 6X SSC, 1X Denhardt's solution, 100 µg/ml yeast tRNA and 0.05% sodium pyrophosphate; and washing of filters at 65° C for 1h in a solution containing 0.1X SSC and 0.1% SDS (sodium dodecyl sulfate).

10 In other embodiments, moderately stringent hybridization conditions are used that are: pretreatment of filters containing nucleic acid for 6 h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA; hybridization for 18-20h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, and 10% (wt/vol) dextran sulfate; followed by washing twice for 1 hour at 55° C in a solution containing 2X SSC and 0.1% SDS.

20 Alternatively, low stringency conditions can be used that are: incubation for 8 hours to overnight at 37° C in a solution comprising 20% formamide, 5 x SSC, 50 mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured sheared salmon sperm DNA; hybridization in the same buffer for 18 to 20 hours; and washing of filters in 1 x SSC at about 37° C for 1 hour.

### **Isolation, Production, Expression, and Mis-expression of MBCAT Nucleic Acids and**

#### **Polypeptides**

25 MBCAT nucleic acids and polypeptides, useful for identifying and testing agents that modulate MBCAT function and for other applications related to the involvement of MBCAT in the beta-catenin pathway. MBCAT nucleic acids and derivatives and orthologs thereof may be obtained using any available method. For instance, techniques for isolating cDNA or genomic DNA sequences of interest by screening DNA libraries or by using polymerase chain reaction (PCR) are well known in the art. In general, the particular use for the protein will dictate the particulars of expression, production, and purification methods. For instance, production of proteins for use in screening for modulating agents may require methods that preserve specific biological activities of these

proteins, whereas production of proteins for antibody generation may require structural integrity of particular epitopes. Expression of proteins to be purified for screening or antibody production may require the addition of specific tags (*e.g.*, generation of fusion proteins). Overexpression of an MBCAT protein for assays used to assess MBCAT function, such as involvement in cell cycle regulation or hypoxic response, may require expression in eukaryotic cell lines capable of these cellular activities. Techniques for the expression, production, and purification of proteins are well known in the art; any suitable means therefore may be used (*e.g.*, Higgins SJ and Hames BD (eds.) *Protein Expression: A Practical Approach*, Oxford University Press Inc., New York 1999; Stanbury PF et al., *Principles of Fermentation Technology*, 2<sup>nd</sup> edition, Elsevier Science, New York, 1995; Doonan S (ed.) *Protein Purification Protocols*, Humana Press, New Jersey, 1996; Coligan JE et al, *Current Protocols in Protein Science* (eds.), 1999, John Wiley & Sons, New York). In particular embodiments, recombinant MBCAT is expressed in a cell line known to have defective beta-catenin function. The recombinant cells are used in cell-based screening assay systems of the invention, as described further below.

The nucleotide sequence encoding an MBCAT polypeptide can be inserted into any appropriate expression vector. The necessary transcriptional and translational signals, including promoter/enhancer element, can derive from the native MBCAT gene and/or its flanking regions or can be heterologous. A variety of host-vector expression systems may be utilized, such as mammalian cell systems infected with virus (*e.g.* vaccinia virus, adenovirus, *etc.*); insect cell systems infected with virus (*e.g.* baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, plasmid, or cosmid DNA. An isolated host cell strain that modulates the expression of, modifies, and/or specifically processes the gene product may be used.

To detect expression of the MBCAT gene product, the expression vector can comprise a promoter operably linked to an MBCAT gene nucleic acid, one or more origins of replication, and, one or more selectable markers (*e.g.* thymidine kinase activity, resistance to antibiotics, *etc.*). Alternatively, recombinant expression vectors can be identified by assaying for the expression of the MBCAT gene product based on the physical or functional properties of the MBCAT protein in *in vitro* assay systems (*e.g.* immunoassays).

The MBCAT protein, fragment, or derivative may be optionally expressed as a fusion, or chimeric protein product (*i.e.* it is joined via a peptide bond to a heterologous protein sequence of a different protein), for example to facilitate purification or detection.

A chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other using standard methods and expressing the chimeric product. A chimeric product may also be made by protein synthetic techniques, *e.g.* by use of a peptide synthesizer (Hunkapiller *et al.*, Nature (1984) 310:105-111).

Once a recombinant cell that expresses the MBCAT gene sequence is identified, the gene product can be isolated and purified using standard methods (*e.g.* ion exchange, affinity, and gel exclusion chromatography; centrifugation; differential solubility; electrophoresis). Alternatively, native MBCAT proteins can be purified from natural sources, by standard methods (*e.g.* immunoaffinity purification). Once a protein is obtained, it may be quantified and its activity measured by appropriate methods, such as immunoassay, bioassay, or other measurements of physical properties, such as crystallography.

The methods of this invention may also use cells that have been engineered for altered expression (mis-expression) of MBCAT or other genes associated with the beta-catenin pathway. As used herein, mis-expression encompasses ectopic expression, over-expression, under-expression, and non-expression (*e.g.* by gene knock-out or blocking expression that would otherwise normally occur).

## **Genetically modified animals**

Animal models that have been genetically modified to alter MBCAT expression may be used in *in vivo* assays to test for activity of a candidate beta-catenin modulating agent, or to further assess the role of MBCAT in a beta-catenin pathway process such as apoptosis or cell proliferation. Preferably, the altered MBCAT expression results in a detectable phenotype, such as decreased or increased levels of cell proliferation, angiogenesis, or apoptosis compared to control animals having normal MBCAT expression. The genetically modified animal may additionally have altered beta-catenin expression (*e.g.* beta-catenin knockout). Preferred genetically modified animals are mammals such as primates, rodents (preferably mice or rats), among others. Preferred non-mammalian species include zebrafish, *C. elegans*, and *Drosophila*. Preferred genetically modified animals are transgenic animals having a heterologous nucleic acid sequence present as an extrachromosomal element in a portion of its cells, *i.e.* mosaic animals (see, for example, techniques described by Jakobovits, 1994, Curr. Biol. 4:761-763.) or stably integrated into its germ line DNA (*i.e.*, in the genomic sequence of most or

all of its cells). Heterologous nucleic acid is introduced into the germ line of such transgenic animals by genetic manipulation of, for example, embryos or embryonic stem cells of the host animal.

Methods of making transgenic animals are well-known in the art (for transgenic mice see Brinster et al., Proc. Nat. Acad. Sci. USA 82: 4438-4442 (1985), U.S. Pat. Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Pat. No. 4,873,191 by Wagner et al., and Hogan, B., Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); for particle bombardment see U.S. Pat. No., 4,945,050, by Sandford *et al.*; for transgenic *Drosophila* see Rubin and Spradling, Science (1982) 218:348-53 and U.S. Pat. No. 4,670,388; for transgenic insects see Berghammer A.J. *et al.*, A Universal Marker for Transgenic Insects (1999) Nature 402:370-371; for transgenic Zebrafish see Lin S., Transgenic Zebrafish, Methods Mol Biol. (2000);136:375-3830); for microinjection procedures for fish, amphibian eggs and birds see Houdebine and Chourrout, Experientia (1991) 47:897-905; for transgenic rats see Hammer *et al.*, Cell (1990) 63:1099-1112; and for culturing of embryonic stem (ES) cells and the subsequent production of transgenic animals by the introduction of DNA into ES cells using methods such as electroporation, calcium phosphate/DNA precipitation and direct injection see, e.g., Teratocarcinomas and Embryonic Stem Cells, A Practical Approach, E. J. Robertson, ed., IRL Press (1987)). Clones of the nonhuman transgenic animals can be produced according to available methods (see Wilmut, I. *et al.* (1997) Nature 385:810-813; and PCT International Publication Nos. WO 97/07668 and WO 97/07669).

In one embodiment, the transgenic animal is a "knock-out" animal having a heterozygous or homozygous alteration in the sequence of an endogenous MBCAT gene that results in a decrease of MBCAT function, preferably such that MBCAT expression is undetectable or insignificant. Knock-out animals are typically generated by homologous recombination with a vector comprising a transgene having at least a portion of the gene to be knocked out. Typically a deletion, addition or substitution has been introduced into the transgene to functionally disrupt it. The transgene can be a human gene (e.g., from a human genomic clone) but more preferably is an ortholog of the human gene derived from the transgenic host species. For example, a mouse MBCAT gene is used to construct a homologous recombination vector suitable for altering an endogenous MBCAT gene in the mouse genome. Detailed methodologies for homologous recombination in mice are available (see Capecchi, Science (1989) 244:1288-1292; Joyner *et al.*, Nature (1989) 338:153-156). Procedures for the production of non-rodent transgenic mammals and other

animals are also available (Houdebine and Chourrout, *supra*; Pursel *et al.*, Science (1989) 244:1281-1288; Simms *et al.*, Bio/Technology (1988) 6:179-183). In a preferred embodiment, knock-out animals, such as mice harboring a knockout of a specific gene, may be used to produce antibodies against the human counterpart of the gene that has been  
5 knocked out (Claesson MH *et al.*, (1994) Scan J Immunol 40:257-264; Declerck PJ *et al.*, (1995) J Biol Chem. 270:8397-400).

In another embodiment, the transgenic animal is a "knock-in" animal having an alteration in its genome that results in altered expression (e.g., increased (including ectopic) or decreased expression) of the MBCAT gene, e.g., by introduction of additional  
10 copies of MBCAT, or by operatively inserting a regulatory sequence that provides for altered expression of an endogenous copy of the MBCAT gene. Such regulatory sequences include inducible, tissue-specific, and constitutive promoters and enhancer elements. The knock-in can be homozygous or heterozygous.

Transgenic nonhuman animals can also be produced that contain selected systems  
15 allowing for regulated expression of the transgene. One example of such a system that may be produced is the cre/loxP recombinase system of bacteriophage P1 (Lakso *et al.*, PNAS (1992) 89:6232-6236; U.S. Pat. No. 4,959,317). If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be  
20 provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) Science 251:1351-1355; U.S. Pat. No. 5,654,182). In a preferred embodiment,  
25 both Cre-LoxP and Flp-Frt are used in the same system to regulate expression of the transgene, and for sequential deletion of vector sequences in the same cell (Sun X *et al.* (2000) Nat Genet 25:83-6).

The genetically modified animals can be used in genetic studies to further elucidate the beta-catenin pathway, as animal models of disease and disorders implicating defective  
30 beta-catenin function, and for *in vivo* testing of candidate therapeutic agents, such as those identified in screens described below. The candidate therapeutic agents are administered to a genetically modified animal having altered MBCAT function and phenotypic changes are compared with appropriate control animals such as genetically modified animals that

receive placebo treatment, and/or animals with unaltered MBCAT expression that receive candidate therapeutic agent.

In addition to the above-described genetically modified animals having altered MBCAT function, animal models having defective beta-catenin function (and otherwise normal MBCAT function), can be used in the methods of the present invention. For example, a beta-catenin knockout mouse can be used to assess, *in vivo*, the activity of a candidate beta-catenin modulating agent identified in one of the *in vitro* assays described below. Preferably, the candidate beta-catenin modulating agent when administered to a model system with cells defective in beta-catenin function, produces a detectable phenotypic change in the model system indicating that the beta-catenin function is restored, i.e., the cells exhibit normal cell cycle progression.

### **Modulating Agents**

The invention provides methods to identify agents that interact with and/or modulate the function of MBCAT and/or the beta-catenin pathway. Modulating agents identified by the methods are also part of the invention. Such agents are useful in a variety of diagnostic and therapeutic applications associated with the beta-catenin pathway, as well as in further analysis of the MBCAT protein and its contribution to the beta-catenin pathway. Accordingly, the invention also provides methods for modulating the beta-catenin pathway comprising the step of specifically modulating MBCAT activity by administering a MBCAT-interacting or -modulating agent.

As used herein, an "MBCAT-modulating agent" is any agent that modulates MBCAT function, for example, an agent that interacts with MBCAT to inhibit or enhance MBCAT activity or otherwise affect normal MBCAT function. MBCAT function can be affected at any level, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In a preferred embodiment, the MBCAT - modulating agent specifically modulates the function of the MBCAT. The phrases "specific modulating agent", "specifically modulates", etc., are used herein to refer to modulating agents that directly bind to the MBCAT polypeptide or nucleic acid, and preferably inhibit, enhance, or otherwise alter, the function of the MBCAT. These phrases also encompasses modulating agents that alter the interaction of the MBCAT with a binding partner, substrate, or cofactor (e.g. by binding to a binding partner of an MBCAT, or to a protein/binding partner complex, and altering MBCAT function). In a further preferred embodiment, the MBCAT- modulating agent is a modulator of the beta-catenin pathway

(e.g. it restores and/or upregulates beta-catenin function) and thus is also a beta-catenin-modulating agent.

Preferred MBCAT-modulating agents include small molecule compounds; MBCAT-interacting proteins, including antibodies and other biotherapeutics; and nucleic acid modulators such as antisense and RNA inhibitors. The modulating agents may be formulated in pharmaceutical compositions, for example, as compositions that may comprise other active ingredients, as in combination therapy, and/or suitable carriers or excipients. Techniques for formulation and administration of the compounds may be found in "Remington's Pharmaceutical Sciences" Mack Publishing Co., Easton, PA, 19<sup>th</sup> edition.

### Small molecule modulators

Small molecules are often preferred to modulate function of proteins with enzymatic function, and/or containing protein interaction domains. Chemical agents, referred to in the art as "small molecule" compounds are typically organic, non-peptide molecules, having a molecular weight less than 10,000, preferably less than 5,000, more preferably less than 1,000, and most preferably less than 500. This class of modulators includes chemically synthesized molecules, for instance, compounds from combinatorial chemical libraries. Synthetic compounds may be rationally designed or identified based on known or inferred properties of the MBCAT protein or may be identified by screening compound libraries. Alternative appropriate modulators of this class are natural products, particularly secondary metabolites from organisms such as plants or fungi, which can also be identified by screening compound libraries for MBCAT-modulating activity. Methods for generating and obtaining compounds are well known in the art (Schreiber SL, Science (2000) 151: 1964-1969; Radmann J and Gunther J, Science (2000) 151:1947-1948).

Small molecule modulators identified from screening assays, as described below, can be used as lead compounds from which candidate clinical compounds may be designed, optimized, and synthesized. Such clinical compounds may have utility in treating pathologies associated with the beta-catenin pathway. The activity of candidate small molecule modulating agents may be improved several-fold through iterative secondary functional validation, as further described below, structure determination, and candidate modulator modification and testing. Additionally, candidate clinical compounds are generated with specific regard to clinical and pharmacological properties. For example,



the reagents may be derivatized and re-screened using *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development.

### Protein Modulators

5           Specific MBCAT-interacting proteins are useful in a variety of diagnostic and therapeutic applications related to the beta-catenin pathway and related disorders, as well as in validation assays for other MBCAT-modulating agents. In a preferred embodiment, MBCAT-interacting proteins affect normal MBCAT function, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In another  
10       embodiment, MBCAT-interacting proteins are useful in detecting and providing information about the function of MBCAT proteins, as is relevant to beta-catenin related disorders, such as cancer (e.g., for diagnostic means).

          An MBCAT-interacting protein may be endogenous, i.e. one that naturally interacts genetically or biochemically with an MBCAT, such as a member of the MBCAT  
15       pathway that modulates MBCAT expression, localization, and/or activity. MBCAT-modulators include dominant negative forms of MBCAT-interacting proteins and of MBCAT proteins themselves. Yeast two-hybrid and variant screens offer preferred methods for identifying endogenous MBCAT-interacting proteins (Finley, R. L. et al. (1996) in DNA Cloning-Expression Systems: A Practical Approach, eds. Glover D. &  
20       Hames B. D (Oxford University Press, Oxford, England), pp. 169-203; Fashema SF et al., Gene (2000) 250:1-14; Drees BL Curr Opin Chem Biol (1999) 3:64-70; Vidal M and Legrain P Nucleic Acids Res (1999) 27:919-29; and U.S. Pat. No. 5,928,868). Mass spectrometry is an alternative preferred method for the elucidation of protein complexes (reviewed in, e.g., Pandley A and Mann M, Nature (2000) 405:837-846; Yates JR 3<sup>rd</sup>,  
25       Trends Genet (2000) 16:5-8).

          An MBCAT-interacting protein may be an exogenous protein, such as an MBCAT-specific antibody or a T-cell antigen receptor (see, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory; Harlow and Lane (1999) Using antibodies: a laboratory manual. Cold Spring Harbor, NY: Cold Spring  
30       Harbor Laboratory Press). MBCAT antibodies are further discussed below.

          In preferred embodiments, an MBCAT-interacting protein specifically binds an MBCAT protein. In alternative preferred embodiments, an MBCAT-modulating agent binds an MBCAT substrate, binding partner, or cofactor.

### *Antibodies*

In another embodiment, the protein modulator is an MBCAT specific antibody agonist or antagonist. The antibodies have therapeutic and diagnostic utilities, and can be used in screening assays to identify MBCAT modulators. The antibodies can also be used  
5 in dissecting the portions of the MBCAT pathway responsible for various cellular responses and in the general processing and maturation of the MBCAT.

Antibodies that specifically bind MBCAT polypeptides can be generated using known methods. Preferably the antibody is specific to a mammalian ortholog of MBCAT polypeptide, and more preferably, to human MBCAT. Antibodies may be polyclonal,  
10 monoclonal (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')<sub>2</sub> fragments, fragments produced by a FAb expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Epitopes of MBCAT which are particularly antigenic can be selected, for example, by routine screening of MBCAT polypeptides for antigenicity or by applying a theoretical  
15 method for selecting antigenic regions of a protein (Hopp and Wood (1981), Proc. Natl. Acad. Sci. U.S.A. 78:3824-28; Hopp and Wood, (1983) Mol. Immunol. 20:483-89; Sutcliffe et al., (1983) Science 219:660-66) to the amino acid sequence shown in any of SEQ ID NOs:10-16. Monoclonal antibodies with affinities of  $10^8 \text{ M}^{-1}$  preferably  $10^9 \text{ M}^{-1}$  to  $10^{10} \text{ M}^{-1}$ , or stronger can be made by standard procedures as described (Harlow and  
20 Lane, *supra*; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed) Academic Press, New York; and U.S. Pat. Nos. 4,381,292; 4,451,570; and 4,618,577). Antibodies may be generated against crude cell extracts of MBCAT or substantially purified fragments thereof. If MBCAT fragments are used, they preferably comprise at least 10, and more preferably, at least 20 contiguous amino acids of an MBCAT protein.  
25 In a particular embodiment, MBCAT-specific antigens and/or immunogens are coupled to carrier proteins that stimulate the immune response. For example, the subject polypeptides are covalently coupled to the keyhole limpet hemocyanin (KLH) carrier, and the conjugate is emulsified in Freund's complete adjuvant, which enhances the immune response. An appropriate immune system such as a laboratory rabbit or mouse is  
30 immunized according to conventional protocols.

The presence of MBCAT-specific antibodies is assayed by an appropriate assay such as a solid phase enzyme-linked immunosorbant assay (ELISA) using immobilized corresponding MBCAT polypeptides. Other assays, such as radioimmunoassays or fluorescent assays might also be used.

Chimeric antibodies specific to MBCAT polypeptides can be made that contain different portions from different animal species. For instance, a human immunoglobulin constant region may be linked to a variable region of a murine mAb, such that the antibody derives its biological activity from the human antibody, and its binding  
5 specificity from the murine fragment. Chimeric antibodies are produced by splicing together genes that encode the appropriate regions from each species (Morrison et al., Proc. Natl. Acad. Sci. (1984) 81:6851-6855; Neuberger et al., Nature (1984) 312:604-608; Takeda et al., Nature (1985) 31:452-454). Humanized antibodies, which are a form of chimeric antibodies, can be generated by grafting complementary-determining regions  
10 (CDRs) (Carlos, T. M., J. M. Harlan. 1994. Blood 84:2068-2101) of mouse antibodies into a background of human framework regions and constant regions by recombinant DNA technology (Riechmann LM, et al., 1988 Nature 323: 323-327). Humanized antibodies contain ~10% murine sequences and ~90% human sequences, and thus further reduce or eliminate immunogenicity, while retaining the antibody specificities (Co MS,  
15 and Queen C. 1991 Nature 351: 501-501; Morrison SL. 1992 Ann. Rev. Immun. 10:239-265). Humanized antibodies and methods of their production are well-known in the art (U.S. Pat. Nos. 5,530,101, 5,585,089, 5,693,762, and 6,180,370).

MBCAT-specific single chain antibodies which are recombinant, single chain polypeptides formed by linking the heavy and light chain fragments of the Fv regions via  
20 an amino acid bridge, can be produced by methods known in the art (U.S. Pat. No. 4,946,778; Bird, Science (1988) 242:423-426; Huston et al., Proc. Natl. Acad. Sci. USA (1988) 85:5879-5883; and Ward et al., Nature (1989) 334:544-546).

Other suitable techniques for antibody production involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of  
25 antibodies in phage or similar vectors (Huse et al., Science (1989) 246:1275-1281). As used herein, T-cell antigen receptors are included within the scope of antibody modulators (Harlow and Lane, 1988, *supra*).

The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, antibodies will be labeled by joining, either covalently  
30 or non-covalently, a substance that provides for a detectable signal, or that is toxic to cells that express the targeted protein (Menard S, et al., Int J. Biol Markers (1989) 4:131-134). A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, fluorescent

emitting lanthanide metals, chemiluminescent moieties, bioluminescent moieties, magnetic particles, and the like (U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241). Also, recombinant immunoglobulins may be produced (U.S. Pat. No. 4,816,567). Antibodies to cytoplasmic polypeptides may be delivered and reach their targets by conjugation with membrane-penetrating toxin proteins (U.S. Pat. No. 6,086,900).

When used therapeutically in a patient, the antibodies of the subject invention are typically administered parenterally, when possible at the target site, or intravenously. The therapeutically effective dose and dosage regimen is determined by clinical studies.

Typically, the amount of antibody administered is in the range of about 0.1 mg/kg –to about 10 mg/kg of patient weight. For parenteral administration, the antibodies are formulated in a unit dosage injectable form (e.g., solution, suspension, emulsion) in association with a pharmaceutically acceptable vehicle. Such vehicles are inherently nontoxic and non-therapeutic. Examples are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils, ethyl oleate, or liposome carriers may also be used. The vehicle may contain minor amounts of additives, such as buffers and preservatives, which enhance isotonicity and chemical stability or otherwise enhance therapeutic potential. The antibodies' concentrations in such vehicles are typically in the range of about 1 mg/ml to about 10 mg/ml.

Immunotherapeutic methods are further described in the literature (US Pat. No. 5,859,206; WO0073469).

### *Specific biotherapeutics*

In a preferred embodiment, an MBCAT-interacting protein may have biotherapeutic applications. Biotherapeutic agents formulated in pharmaceutically acceptable carriers and dosages may be used to activate or inhibit signal transduction pathways. This modulation may be accomplished by binding a ligand, thus inhibiting the activity of the pathway; or by binding a receptor, either to inhibit activation of, or to activate, the receptor. Alternatively, the biotherapeutic may itself be a ligand capable of activating or inhibiting a receptor. Biotherapeutic agents and methods of producing them are described in detail in U.S. Pat. No. 6,146,628.

When the MBCAT is a ligand, it may be used as a biotherapeutic agent to activate or inhibit its natural receptor. Alternatively, antibodies against MBCAT, as described in the previous section, may be used as biotherapeutic agents.

When the MBCAT is a receptor, its ligand(s), antibodies to the ligand(s) or the MBCAT itself may be used as biotherapeutics to modulate the activity of MBCAT in the beta-catenin pathway.

## 5            **Nucleic Acid Modulators**

Other preferred MBCAT-modulating agents comprise nucleic acid molecules, such as antisense oligomers or double stranded RNA (dsRNA), which generally inhibit MBCAT activity. Preferred nucleic acid modulators interfere with the function of the MBCAT nucleic acid such as DNA replication, transcription, translocation of the MBCAT  
10 RNA to the site of protein translation, translation of protein from the MBCAT RNA, splicing of the MBCAT RNA to yield one or more mRNA species, or catalytic activity which may be engaged in or facilitated by the MBCAT RNA.

In one embodiment, the antisense oligomer is an oligonucleotide that is sufficiently complementary to an MBCAT mRNA to bind to and prevent translation, preferably by  
15 binding to the 5' untranslated region. MBCAT-specific antisense oligonucleotides, preferably range from at least 6 to about 200 nucleotides. In some embodiments the oligonucleotide is preferably at least 10, 15, or 20 nucleotides in length. In other embodiments, the oligonucleotide is preferably less than 50, 40, or 30 nucleotides in length. The oligonucleotide can be DNA or RNA or a chimeric mixture or derivatives or  
20 modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, agents that facilitate transport across the cell membrane, hybridization-triggered cleavage agents, and intercalating agents.

25 In another embodiment, the antisense oligomer is a phosphothioate morpholino oligomer (PMO). PMOs are assembled from four different morpholino subunits, each of which contain one of four genetic bases (A, C, G, or T) linked to a six-membered morpholine ring. Polymers of these subunits are joined by non-ionic phosphoramidate intersubunit linkages. Details of how to make and use PMOs and other antisense  
30 oligomers are well known in the art (e.g. see WO99/18193; Probst JC, Antisense Oligodeoxynucleotide and Ribozyme Design, Methods. (2000) 22(3):271-281; Summerton J, and Weller D. 1997 Antisense Nucleic Acid Drug Dev. :7:187-95; US Pat. No. 5,235,033; and US Pat No. 5,378,841).

Alternative preferred MBCAT nucleic acid modulators are double-stranded RNA species mediating RNA interference (RNAi). RNAi is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. Methods relating to the use of RNAi to silence genes in *C. elegans*, *Drosophila*, plants, and humans are known in the art (Fire A, et al., 1998 Nature 391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); Sharp, P. A. RNA interference 2001. Genes Dev. 15, 485-490 (2001); Hammond, S. M., et al., Nature Rev. Genet. 2, 110-1119 (2001); Tuschl, T. Chem. Biochem. 2, 239-245 (2001); Hamilton, A. et al., Science 286, 950-952 (1999); Hammond, S. M., et al., Nature 404, 293-296 (2000); Zamore, P. D., et al., Cell 101, 25-33 (2000); Bernstein, E., et al., Nature 409, 363-366 (2001); Elbashir, S. M., et al., Genes Dev. 15, 188-200 (2001); WO0129058; WO9932619; Elbashir SM, et al., 2001 Nature 411:494-498).

Nucleic acid modulators are commonly used as research reagents, diagnostics, and therapeutics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used to elucidate the function of particular genes (see, for example, U.S. Pat. No. 6,165,790). Nucleic acid modulators are also used, for example, to distinguish between functions of various members of a biological pathway. For example, antisense oligomers have been employed as therapeutic moieties in the treatment of disease states in animals and man and have been demonstrated in numerous clinical trials to be safe and effective (Milligan JF, et al., Current Concepts in Antisense Drug Design, J Med Chem. (1993) 36:1923-1937; Tonkinson JL et al., Antisense Oligodeoxynucleotides as Clinical Therapeutic Agents, Cancer Invest. (1996) 14:54-65). Accordingly, in one aspect of the invention, an MBCAT-specific nucleic acid modulator is used in an assay to further elucidate the role of the MBCAT in the beta-catenin pathway, and/or its relationship to other members of the pathway. In another aspect of the invention, an MBCAT-specific antisense oligomer is used as a therapeutic agent for treatment of beta-catenin-related disease states.

### Assay Systems

The invention provides assay systems and screening methods for identifying specific modulators of MBCAT activity. As used herein, an "assay system" encompasses all the components required for performing and analyzing results of an assay that detects and/or measures a particular event. In general, primary assays are used to identify or confirm a modulator's specific biochemical or molecular effect with respect to the

MBCAT nucleic acid or protein. In general, secondary assays further assess the activity of a MBCAT modulating agent identified by a primary assay and may confirm that the modulating agent affects MBCAT in a manner relevant to the beta-catenin pathway. In some cases, MBCAT modulators will be directly tested in a secondary assay.

5           In a preferred embodiment, the screening method comprises contacting a suitable assay system comprising an MBCAT polypeptide or nucleic acid with a candidate agent under conditions whereby, but for the presence of the agent, the system provides a reference activity (e.g. binding activity), which is based on the particular molecular event the screening method detects. A statistically significant difference between the agent-  
10       biased activity and the reference activity indicates that the candidate agent modulates MBCAT activity, and hence the beta-catenin pathway. The MBCAT polypeptide or nucleic acid used in the assay may comprise any of the nucleic acids or polypeptides described above.

## 15           **Primary Assays**

The type of modulator tested generally determines the type of primary assay.

### *Primary assays for small molecule modulators*

For small molecule modulators, screening assays are used to identify candidate  
20       modulators. Screening assays may be cell-based or may use a cell-free system that recreates or retains the relevant biochemical reaction of the target protein (reviewed in Sittampalam GS *et al.*, Curr Opin Chem Biol (1997) 1:384-91 and accompanying references). As used herein the term "cell-based" refers to assays using live cells, dead cells, or a particular cellular fraction, such as a membrane, endoplasmic reticulum, or  
25       mitochondrial fraction. The term "cell free" encompasses assays using substantially purified protein (either endogenous or recombinantly produced), partially purified or crude cellular extracts. Screening assays may detect a variety of molecular events, including protein-DNA interactions, protein-protein interactions (e.g., receptor-ligand binding), transcriptional activity (e.g., using a reporter gene), enzymatic activity (e.g., via a property  
30       of the substrate), activity of second messengers, immunogenicity and changes in cellular morphology or other cellular characteristics. Appropriate screening assays may use a wide range of detection methods including fluorescent, radioactive, colorimetric, spectrophotometric, and amperometric methods, to provide a read-out for the particular molecular event detected.

Cell-based screening assays usually require systems for recombinant expression of MBCAT and any auxiliary proteins demanded by the particular assay. Appropriate methods for generating recombinant proteins produce sufficient quantities of proteins that retain their relevant biological activities and are of sufficient purity to optimize activity and assure assay reproducibility. Yeast two-hybrid and variant screens, and mass spectrometry provide preferred methods for determining protein-protein interactions and elucidation of protein complexes. In certain applications, when MBCAT-interacting proteins are used in screens to identify small molecule modulators, the binding specificity of the interacting protein to the MBCAT protein may be assayed by various known methods such as substrate processing (e.g. ability of the candidate MBCAT-specific binding agents to function as negative effectors in MBCAT-expressing cells), binding equilibrium constants (usually at least about  $10^7 \text{ M}^{-1}$ , preferably at least about  $10^8 \text{ M}^{-1}$ , more preferably at least about  $10^9 \text{ M}^{-1}$ ), and immunogenicity (e.g. ability to elicit MBCAT specific antibody in a heterologous host such as a mouse, rat, goat or rabbit). For enzymes and receptors, binding may be assayed by, respectively, substrate and ligand processing.

The screening assay may measure a candidate agent's ability to specifically bind to or modulate activity of a MBCAT polypeptide, a fusion protein thereof, or to cells or membranes bearing the polypeptide or fusion protein. The MBCAT polypeptide can be full length or a fragment thereof that retains functional MBCAT activity. The MBCAT polypeptide may be fused to another polypeptide, such as a peptide tag for detection or anchoring, or to another tag. The MBCAT polypeptide is preferably human MBCAT, or is an ortholog or derivative thereof as described above. In a preferred embodiment, the screening assay detects candidate agent-based modulation of MBCAT interaction with a binding target, such as an endogenous or exogenous protein or other substrate that has MBCAT-specific binding activity, and can be used to assess normal MBCAT gene function.

Suitable assay formats that may be adapted to screen for MBCAT modulators are known in the art. Preferred screening assays are high throughput or ultra high throughput and thus provide automated, cost-effective means of screening compound libraries for lead compounds (Fernandes PB, Curr Opin Chem Biol (1998) 2:597-603; Sundberg SA, Curr Opin Biotechnol 2000, 11:47-53). In one preferred embodiment, screening assays uses fluorescence technologies, including fluorescence polarization, time-resolved fluorescence, and fluorescence resonance energy transfer. These systems offer means to monitor protein-protein or DNA-protein interactions in which the intensity of the signal



emitted from dye-labeled molecules depends upon their interactions with partner molecules (*e.g.*, Selvin PR, Nat Struct Biol (2000) 7:730-4; Fernandes PB, *supra*; Hertzberg RP and Pope AJ, Curr Opin Chem Biol (2000) 4:445-451).

5 A variety of suitable assay systems may be used to identify candidate MBCAT and beta-catenin pathway modulators (*e.g.* U.S. Pat. No. 6,165,992 (kinase assays); U.S. Pat. Nos. 5,550,019 and 6,133,437 (apoptosis assays); U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434 (angiogenesis assays), among others). Specific preferred assays are described in more detail below.

10 Protein kinases, key signal transduction proteins that may be either membrane-associated or intracellular, catalyze the transfer of gamma phosphate from adenosine triphosphate (ATP) to a serine, threonine or tyrosine residue in a protein substrate. Radioassays, which monitor the transfer from [ $\gamma$ - $^{32}\text{P}$  or  $^{33}\text{P}$ ]ATP, are frequently used to assay kinase activity. For instance, a scintillation assay for p56 (lck) kinase activity monitors the transfer of the gamma phosphate from [ $\gamma$ - $^{33}\text{P}$ ] ATP to a  
15 biotinylated peptide substrate. The substrate is captured on a streptavidin coated bead that transmits the signal (Beveridge M *et al.*, J Biomol Screen (2000) 5:205-212). This assay uses the scintillation proximity assay (SPA), in which only radio-ligand bound to receptors tethered to the surface of an SPA bead are detected by the scintillant immobilized within it, allowing binding to be measured without separation of bound from free ligand. Other  
20 assays for protein kinase activity may use antibodies that specifically recognize phosphorylated substrates. For instance, the kinase receptor activation (KIRA) assay measures receptor tyrosine kinase activity by ligand stimulating the intact receptor in cultured cells, then capturing solubilized receptor with specific antibodies and quantifying phosphorylation via phosphotyrosine ELISA (Sadick MD, Dev Biol Stand (1999) 97:121-  
25 133). Another example of antibody based assays for protein kinase activity is TRF (time-resolved fluorometry). This method utilizes europium chelate-labeled anti-phosphotyrosine antibodies to detect phosphate transfer to a polymeric substrate coated onto microtiter plate wells. The amount of phosphorylation is then detected using time-resolved, dissociation-enhanced fluorescence (Braunwalder AF, *et al.*, Anal Biochem 1996  
30 Jul 1;238(2):159-64).

Adapter proteins are involved in a wide range of signaling and other cellular processes and generally facilitate protein-protein or protein-nucleic acid interactions via certain conserved motifs, including PDZ, SH2, SH3, PH, TRAF, WD40, LIM, ankyrin repeat, KH and annexin domains, etc. Assays for adapter protein activity may measure

protein binding at the conserved motifs. For instance, exemplary assays for SH2 domain-containing proteins have measured binding using fluorescently labeled peptide substrate and fluorescence polarization or laser-scanning techniques (Lynch BA et al., Anal Biochem 1999, 275:62-73; Zuck P et al., Proc Natl Acad Sci USA 1999, 96: 11122-11127). An alternative SH2 binding assay uses radiolabeled peptide. An assay for protein-protein interaction at the LIM domain has used fluorescently labeled LIM-containing proteins (FHL2 and FHL3) and the fluorescence resonance energy transfer (FRET) technique (Li HY, J Cell Biochem 2001, 80:293-303).

10           **Apoptosis assays.** Assays for apoptosis may be performed by terminal deoxynucleotidyl transferase-mediated digoxigenin-11-dUTP nick end labeling (TUNEL) assay. The TUNEL assay is used to measure nuclear DNA fragmentation characteristic of apoptosis (Lazebnik *et al.*, 1994, Nature 371, 346), by following the incorporation of fluorescein-dUTP (Yonehara *et al.*, 1989, J. Exp. Med. 169, 1747). Apoptosis may further  
15 be assayed by acridine orange staining of tissue culture cells (Lucas, R., et al., 1998, Blood 15:4730-41). An apoptosis assay system may comprise a cell that expresses an MBCAT, and that optionally has defective beta-catenin function (e.g. beta-catenin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the apoptosis  
20 assay system and changes in induction of apoptosis relative to controls where no test agent is added, identify candidate beta-catenin modulating agents. In some embodiments of the invention, an apoptosis assay may be used as a secondary assay to test a candidate beta-catenin modulating agents that is initially identified using a cell-free assay system. An apoptosis assay may also be used to test whether MBCAT function plays a direct role in  
25 apoptosis. For example, an apoptosis assay may be performed on cells that over- or under-express MBCAT relative to wild type cells. Differences in apoptotic response compared to wild type cells suggests that the MBCAT plays a direct role in the apoptotic response. Apoptosis assays are described further in US Pat. No. 6,133,437.

**Cell proliferation and cell cycle assays.** Cell proliferation may be assayed via  
30 bromodeoxyuridine (BRDU) incorporation. This assay identifies a cell population undergoing DNA synthesis by incorporation of BRDU into newly-synthesized DNA. Newly-synthesized DNA may then be detected using an anti-BRDU antibody (Hoshino *et al.*, 1986, Int. J. Cancer 38, 369; Campana *et al.*, 1988, J. Immunol. Meth. 107, 79), or by other means.

Cell Proliferation may also be examined using [ $^3\text{H}$ ]-thymidine incorporation (Chen, J., 1996, *Oncogene* 13:1395-403; Jeoung, J., 1995, *J. Biol. Chem.* 270:18367-73). This assay allows for quantitative characterization of S-phase DNA syntheses. In this assay, cells synthesizing DNA will incorporate [ $^3\text{H}$ ]-thymidine into newly synthesized DNA. Incorporation can then be measured by standard techniques such as by counting of radioisotope in a scintillation counter (e.g., Beckman LS 3800 Liquid Scintillation Counter). Another proliferation assay uses the dye Alamar Blue (available from Biosource International), which fluoresces when reduced in living cells and provides an indirect measurement of cell number (Voytik-Harbin SL et al., 1998, *In Vitro Cell Dev Biol Anim* 34:239-46).

Cell proliferation may also be assayed by colony formation in soft agar (Sambrook et al., *Molecular Cloning*; Cold Spring Harbor (1989)). For example, cells transformed with MBCAT are seeded in soft agar plates, and colonies are measured and counted after two weeks incubation.

Involvement of a gene in the cell cycle may be assayed by flow cytometry (Gray JW et al. (1986) *Int J Radiat Biol Relat Stud Phys Chem Med* 49:237-55). Cells transfected with an MBCAT may be stained with propidium iodide and evaluated in a flow cytometer (available from Becton Dickinson), which indicates accumulation of cells in different stages of the cell cycle.

Accordingly, a cell proliferation or cell cycle assay system may comprise a cell that expresses an MBCAT, and that optionally has defective beta-catenin function (e.g. beta-catenin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the assay system and changes in cell proliferation or cell cycle relative to controls where no test agent is added, identify candidate beta-catenin modulating agents.

In some embodiments of the invention, the cell proliferation or cell cycle assay may be used as a secondary assay to test a candidate beta-catenin modulating agents that is initially identified using another assay system such as a cell-free assay system. A cell proliferation assay may also be used to test whether MBCAT function plays a direct role in cell proliferation or cell cycle. For example, a cell proliferation or cell cycle assay may be performed on cells that over- or under-express MBCAT relative to wild type cells. Differences in proliferation or cell cycle compared to wild type cells suggests that the MBCAT plays a direct role in cell proliferation or cell cycle.

**Angiogenesis.** Angiogenesis may be assayed using various human endothelial cell systems, such as umbilical vein, coronary artery, or dermal cells. Suitable assays include Alamar Blue based assays (available from Biosource International) to measure proliferation; migration assays using fluorescent molecules, such as the use of Becton Dickinson Falcon HTS FluoroBlock cell culture inserts to measure migration of cells through membranes in presence or absence of angiogenesis enhancer or suppressors; and tubule formation assays based on the formation of tubular structures by endothelial cells on Matrigel® (Becton Dickinson). Accordingly, an angiogenesis assay system may comprise a cell that expresses an MBCAT, and that optionally has defective beta-catenin function (e.g. beta-catenin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the angiogenesis assay system and changes in angiogenesis relative to controls where no test agent is added, identify candidate beta-catenin modulating agents. In some embodiments of the invention, the angiogenesis assay may be used as a secondary assay to test a candidate beta-catenin modulating agents that is initially identified using another assay system. An angiogenesis assay may also be used to test whether MBCAT function plays a direct role in cell proliferation. For example, an angiogenesis assay may be performed on cells that over- or under-express MBCAT relative to wild type cells. Differences in angiogenesis compared to wild type cells suggests that the MBCAT plays a direct role in angiogenesis. U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434, among others.

**Hypoxic induction.** The alpha subunit of the transcription factor, hypoxia inducible factor-1 (HIF-1), is upregulated in tumor cells following exposure to hypoxia in vitro. Under hypoxic conditions, HIF-1 stimulates the expression of genes known to be important in tumour cell survival, such as those encoding glycolytic enzymes and VEGF. Induction of such genes by hypoxic conditions may be assayed by growing cells transfected with MBCAT in hypoxic conditions (such as with 0.1% O<sub>2</sub>, 5% CO<sub>2</sub>, and balance N<sub>2</sub>, generated in a Napco 7001 incubator (Precision Scientific)) and normoxic conditions, followed by assessment of gene activity or expression by Taqman®. For example, a hypoxic induction assay system may comprise a cell that expresses an MBCAT, and that optionally has defective beta-catenin function (e.g. beta-catenin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the hypoxic induction assay system and changes in hypoxic response relative to controls where no test agent is added, identify candidate beta-catenin modulating agents. In some

embodiments of the invention, the hypoxic induction assay may be used as a secondary assay to test a candidate beta-catenin modulating agents that is initially identified using another assay system. A hypoxic induction assay may also be used to test whether MBCAT function plays a direct role in the hypoxic response. For example, a hypoxic induction assay may be performed on cells that over- or under-express MBCAT relative to wild type cells. Differences in hypoxic response compared to wild type cells suggests that the MBCAT plays a direct role in hypoxic induction.

**Cell adhesion.** Cell adhesion assays measure adhesion of cells to purified adhesion proteins, or adhesion of cells to each other, in presence or absence of candidate modulating agents. Cell-protein adhesion assays measure the ability of agents to modulate the adhesion of cells to purified proteins. For example, recombinant proteins are produced, diluted to 2.5g/mL in PBS, and used to coat the wells of a microtiter plate. The wells used for negative control are not coated. Coated wells are then washed, blocked with 1% BSA, and washed again. Compounds are diluted to 2× final test concentration and added to the blocked, coated wells. Cells are then added to the wells, and the unbound cells are washed off. Retained cells are labeled directly on the plate by adding a membrane-permeable fluorescent dye, such as calcein-AM, and the signal is quantified in a fluorescent microplate reader.

Cell-cell adhesion assays measure the ability of agents to modulate binding of cell adhesion proteins with their native ligands. These assays use cells that naturally or recombinantly express the adhesion protein of choice. In an exemplary assay, cells expressing the cell adhesion protein are plated in wells of a multiwell plate. Cells expressing the ligand are labeled with a membrane-permeable fluorescent dye, such as BCECF, and allowed to adhere to the monolayers in the presence of candidate agents. Unbound cells are washed off, and bound cells are detected using a fluorescence plate reader.

High-throughput cell adhesion assays have also been described. In one such assay, small molecule ligands and peptides are bound to the surface of microscope slides using a microarray spotter, intact cells are then contacted with the slides, and unbound cells are washed off. In this assay, not only the binding specificity of the peptides and modulators against cell lines are determined, but also the functional cell signaling of attached cells using immunofluorescence techniques in situ on the microchip is measured (Falsey JR et al., Bioconj Chem. 2001 May-Jun;12(3):346-53).

**Tubulogenesis.** Tubulogenesis assays monitor the ability of cultured cells, generally endothelial cells, to form tubular structures on a matrix substrate, which generally simulates the environment of the extracellular matrix. Exemplary substrates include Matrigel™ (Becton Dickinson), an extract of basement membrane proteins containing laminin, collagen IV, and heparin sulfate proteoglycan, which is liquid at 4° C and forms a solid gel at 37° C. Other suitable matrices comprise extracellular components such as collagen, fibronectin, and/or fibrin. Cells are stimulated with a pro-angiogenic stimulant, and their ability to form tubules is detected by imaging. Tubules can generally be detected after an overnight incubation with stimuli, but longer or shorter time frames may also be used. Tube formation assays are well known in the art (e.g., Jones MK et al., 1999, Nature Medicine 5:1418-1423). These assays have traditionally involved stimulation with serum or with the growth factors FGF or VEGF. Serum represents an undefined source of growth factors. In a preferred embodiment, the assay is performed with cells cultured in serum free medium, in order to control which process or pathway a candidate agent modulates. Moreover, we have found that different target genes respond differently to stimulation with different pro-angiogenic agents, including inflammatory angiogenic factors such as TNF- $\alpha$ . Thus, in a further preferred embodiment, a tubulogenesis assay system comprises testing an MBCAT's response to a variety of factors, such as FGF, VEGF, phorbol myristate acetate (PMA), TNF- $\alpha$ , ephrin, etc.

20

**Cell Migration.** An invasion/migration assay (also called a migration assay) tests the ability of cells to overcome a physical barrier and to migrate towards pro-angiogenic signals. Migration assays are known in the art (e.g., Paik JH et al., 2001, J Biol Chem 276:11830-11837). In a typical experimental set-up, cultured endothelial cells are seeded onto a matrix-coated porous lamina, with pore sizes generally smaller than typical cell size. The matrix generally simulates the environment of the extracellular matrix, as described above. The lamina is typically a membrane, such as the transwell polycarbonate membrane (Corning Costar Corporation, Cambridge, MA), and is generally part of an upper chamber that is in fluid contact with a lower chamber containing pro-angiogenic stimuli. Migration is generally assayed after an overnight incubation with stimuli, but longer or shorter time frames may also be used. Migration is assessed as the number of cells that crossed the lamina, and may be detected by staining cells with hemotoxylin solution (VWR Scientific, South San Francisco, CA), or by any other method for determining cell number. In another exemplary set up, cells are fluorescently labeled and

30

migration is detected using fluorescent readings, for instance using the Falcon HTS FluoroBlok (Becton Dickinson). While some migration is observed in the absence of stimulus, migration is greatly increased in response to pro-angiogenic factors. As described above, a preferred assay system for migration/invasion assays comprises testing an MBCAT's response to a variety of pro-angiogenic factors, including tumor angiogenic and inflammatory angiogenic agents, and culturing the cells in serum free medium.

**Sprouting assay.** A sprouting assay is a three-dimensional *in vitro* angiogenesis assay that uses a cell-number defined spheroid aggregation of endothelial cells ("spheroid"), embedded in a collagen gel-based matrix. The spheroid can serve as a starting point for the sprouting of capillary-like structures by invasion into the extracellular matrix (termed "cell sprouting") and the subsequent formation of complex anastomosing networks (Korff and Augustin, 1999, J Cell Sci 112:3249-58). In an exemplary experimental set-up, spheroids are prepared by pipetting 400 human umbilical vein endothelial cells into individual wells of a nonadhesive 96-well plates to allow overnight spheroidal aggregation (Korff and Augustin: J Cell Biol 143: 1341-52, 1998). Spheroids are harvested and seeded in 900 $\mu$ l of methocel-collagen solution and pipetted into individual wells of a 24 well plate to allow collagen gel polymerization. Test agents are added after 30 min by pipetting 100  $\mu$ l of 10-fold concentrated working dilution of the test substances on top of the gel. Plates are incubated at 37°C for 24h. Dishes are fixed at the end of the experimental incubation period by addition of paraformaldehyde. Sprouting intensity of endothelial cells can be quantitated by an automated image analysis system to determine the cumulative sprout length per spheroid.

#### 25 ***Primary assays for antibody modulators***

For antibody modulators, appropriate primary assays test is a binding assay that tests the antibody's affinity to and specificity for the MBCAT protein. Methods for testing antibody affinity and specificity are well known in the art (Harlow and Lane, 1988, 1999, *supra*). The enzyme-linked immunosorbant assay (ELISA) is a preferred method for detecting MBCAT-specific antibodies; others include FACS assays, radioimmunoassays, and fluorescent assays.

In some cases, screening assays described for small molecule modulators may also be used to test antibody modulators.

### *Primary assays for nucleic acid modulators*

For nucleic acid modulators, primary assays may test the ability of the nucleic acid modulator to inhibit or enhance MBCAT gene expression, preferably mRNA expression. In general, expression analysis comprises comparing MBCAT expression in like populations of cells (*e.g.*, two pools of cells that endogenously or recombinantly express MBCAT) in the presence and absence of the nucleic acid modulator. Methods for analyzing mRNA and protein expression are well known in the art. For instance, Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR (*e.g.*, using the TaqMan®, PE Applied Biosystems), or microarray analysis may be used to confirm that MBCAT mRNA expression is reduced in cells treated with the nucleic acid modulator (*e.g.*, Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, eds., John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm DH and Guiseppi-Elie, A Curr Opin Biotechnol 2001, 12:41-47). Protein expression may also be monitored. Proteins are most commonly detected with specific antibodies or antisera directed against either the MBCAT protein or specific peptides. A variety of means including Western blotting, ELISA, or in situ detection, are available (Harlow E and Lane D, 1988 and 1999, *supra*).

In some cases, screening assays described for small molecule modulators, particularly in assay systems that involve MBCAT mRNA expression, may also be used to test nucleic acid modulators.

### **Secondary Assays**

Secondary assays may be used to further assess the activity of MBCAT-modulating agent identified by any of the above methods to confirm that the modulating agent affects MBCAT in a manner relevant to the beta-catenin pathway. As used herein, MBCAT-modulating agents encompass candidate clinical compounds or other agents derived from previously identified modulating agent. Secondary assays can also be used to test the activity of a modulating agent on a particular genetic or biochemical pathway or to test the specificity of the modulating agent's interaction with MBCAT.

Secondary assays generally compare like populations of cells or animals (*e.g.*, two pools of cells or animals that endogenously or recombinantly express MBCAT) in the presence and absence of the candidate modulator. In general, such assays test whether treatment of cells or animals with a candidate MBCAT-modulating agent results in changes in the beta-catenin pathway in comparison to untreated (or mock- or placebo-



treated) cells or animals. Certain assays use "sensitized genetic backgrounds", which, as used herein, describe cells or animals engineered for altered expression of genes in the beta-catenin or interacting pathways.

### 5           *Cell-based assays*

Cell based assays may detect endogenous beta-catenin pathway activity or may rely on recombinant expression of beta-catenin pathway components. Any of the aforementioned assays may be used in this cell-based format. Candidate modulators are typically added to the cell media but may also be injected into cells or delivered by any  
10   other efficacious means.

### *Animal Assays*

A variety of non-human animal models of normal or defective beta-catenin pathway may be used to test candidate MBCAT modulators. Models for defective beta-  
15   catenin pathway typically use genetically modified animals that have been engineered to mis-express (*e.g.*, over-express or lack expression in) genes involved in the beta-catenin pathway. Assays generally require systemic delivery of the candidate modulators, such as by oral administration, injection, etc.

In a preferred embodiment, beta-catenin pathway activity is assessed by  
20   monitoring neovascularization and angiogenesis. Animal models with defective and normal beta-catenin are used to test the candidate modulator's affect on MBCAT in Matrigel® assays. Matrigel® is an extract of basement membrane proteins, and is composed primarily of laminin, collagen IV, and heparin sulfate proteoglycan. It is provided as a sterile liquid at 4° C, but rapidly forms a solid gel at 37° C. Liquid  
25   Matrigel® is mixed with various angiogenic agents, such as bFGF and VEGF, or with human tumor cells which over-express the MBCAT. The mixture is then injected subcutaneously(SC) into female athymic nude mice (Taconic, Germantown, NY) to support an intense vascular response. Mice with Matrigel® pellets may be dosed via oral (PO), intraperitoneal (IP), or intravenous (IV) routes with the candidate modulator. Mice  
30   are euthanized 5 - 12 days post-injection, and the Matrigel® pellet is harvested for hemoglobin analysis (Sigma plasma hemoglobin kit). Hemoglobin content of the gel is found to correlate the degree of neovascularization in the gel.

In another preferred embodiment, the effect of the candidate modulator on MBCAT is assessed via tumorigenicity assays. Tumor xenograft assays are known in the

art (see, e.g., Ogawa K et al., 2000, Oncogene 19:6043-6052). Xenografts are typically implanted SC into female athymic mice, 6-7 week old, as single cell suspensions either from a pre-existing tumor or from *in vitro* culture. The tumors which express the MBCAT endogenously are injected in the flank,  $1 \times 10^5$  to  $1 \times 10^7$  cells per mouse in a volume of 100  $\mu$ L using a 27gauge needle. Mice are then ear tagged and tumors are measured twice weekly. Candidate modulator treatment is initiated on the day the mean tumor weight reaches 100 mg. Candidate modulator is delivered IV, SC, IP, or PO by bolus administration. Depending upon the pharmacokinetics of each unique candidate modulator, dosing can be performed multiple times per day. The tumor weight is assessed by measuring perpendicular diameters with a caliper and calculated by multiplying the measurements of diameters in two dimensions. At the end of the experiment, the excised tumors maybe utilized for biomarker identification or further analyses. For immunohistochemistry staining, xenograft tumors are fixed in 4% paraformaldehyde, 0.1M phosphate, pH 7.2, for 6 hours at 4°C, immersed in 30% sucrose in PBS, and rapidly frozen in isopentane cooled with liquid nitrogen.

In another preferred embodiment, tumorigenicity is monitored using a hollow fiber assay, which is described in U.S. Pat No. US 5,698,413. Briefly, the method comprises implanting into a laboratory animal a biocompatible, semi-permeable encapsulation device containing target cells, treating the laboratory animal with a candidate modulating agent, and evaluating the target cells for reaction to the candidate modulator. Implanted cells are generally human cells from a pre-existing tumor or a tumor cell line. After an appropriate period of time, generally around six days, the implanted samples are harvested for evaluation of the candidate modulator. Tumorigenicity and modulator efficacy may be evaluated by assaying the quantity of viable cells present in the macrocapsule, which can be determined by tests known in the art, for example, MTT dye conversion assay, neutral red dye uptake, trypan blue staining, viable cell counts, the number of colonies formed in soft agar, the capacity of the cells to recover and replicate *in vitro*, etc.

In another preferred embodiment, a tumorigenicity assay use a transgenic animal, usually a mouse, carrying a dominant oncogene or tumor suppressor gene knockout under the control of tissue specific regulatory sequences; these assays are generally referred to as transgenic tumor assays. In a preferred application, tumor development in the transgenic model is well characterized or is controlled. In an exemplary model, the "RIP1-Tag2" transgene, comprising the SV40 large T-antigen oncogene under control of the insulin gene regulatory regions is expressed in pancreatic beta cells and results in islet cell

carcinomas (Hanahan D, 1985, Nature 315:115-122; Parangi S et al, 1996, Proc Natl Acad Sci USA 93: 2002-2007; Bergers G et al, 1999, Science 284:808-812). An "angiogenic switch," occurs at approximately five weeks, as normally quiescent capillaries in a subset of hyperproliferative islets become angiogenic. The RIP1-TAG2 mice die by age 14 weeks. Candidate modulators may be administered at a variety of stages, including just prior to the angiogenic switch (e.g., for a model of tumor prevention), during the growth of small tumors (e.g., for a model of intervention), or during the growth of large and/or invasive tumors (e.g., for a model of regression). Tumorogenicity and modulator efficacy can be evaluating life-span extension and/or tumor characteristics, including number of tumors, tumor size, tumor morphology, vessel density, apoptotic index, etc.

### **Diagnostic and therapeutic uses**

Specific MBCAT-modulating agents are useful in a variety of diagnostic and therapeutic applications where disease or disease prognosis is related to defects in the beta-catenin pathway, such as angiogenic, apoptotic, or cell proliferation disorders. Accordingly, the invention also provides methods for modulating the beta-catenin pathway in a cell, preferably a cell pre-determined to have defective or impaired beta-catenin function (e.g. due to overexpression, underexpression, or misexpression of beta-catenin, or due to gene mutations), comprising the step of administering an agent to the cell that specifically modulates MBCAT activity. Preferably, the modulating agent produces a detectable phenotypic change in the cell indicating that the beta-catenin function is restored. The phrase "function is restored", and equivalents, as used herein, means that the desired phenotype is achieved, or is brought closer to normal compared to untreated cells. For example, with restored beta-catenin function, cell proliferation and/or progression through cell cycle may normalize, or be brought closer to normal relative to untreated cells. The invention also provides methods for treating disorders or disease associated with impaired beta-catenin function by administering a therapeutically effective amount of an MBCAT -modulating agent that modulates the beta-catenin pathway. The invention further provides methods for modulating MBCAT function in a cell, preferably a cell pre-determined to have defective or impaired MBCAT function, by administering an MBCAT -modulating agent. Additionally, the invention provides a method for treating disorders or disease associated with impaired MBCAT function by administering a therapeutically effective amount of an MBCAT -modulating agent.

The discovery that MBCAT is implicated in beta-catenin pathway provides for a variety of methods that can be employed for the diagnostic and prognostic evaluation of diseases and disorders involving defects in the beta-catenin pathway and for the identification of subjects having a predisposition to such diseases and disorders.

5 Various expression analysis methods can be used to diagnose whether MBCAT expression occurs in a particular sample, including Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR, and microarray analysis. (*e.g.*, Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, *eds.*, John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, *Biotechniques* (1999) 26:112-125; Kallioniemi OP, *Ann*  
10 *Med* 2001, 33:142-147; Blohm and Guiseppi-Elie, *Curr Opin Biotechnol* 2001, 12:41-47). Tissues having a disease or disorder implicating defective beta-catenin signaling that express an MBCAT, are identified as amenable to treatment with an MBCAT modulating agent. In a preferred application, the beta-catenin defective tissue overexpresses an MBCAT relative to normal tissue. For example, a Northern blot analysis of mRNA from  
15 tumor and normal cell lines, or from tumor and matching normal tissue samples from the same patient, using full or partial MBCAT cDNA sequences as probes, can determine whether particular tumors express or overexpress MBCAT. Alternatively, the TaqMan® is used for quantitative RT-PCR analysis of MBCAT expression in cell lines, normal tissues and tumor samples (PE Applied Biosystems).

20 Various other diagnostic methods may be performed, for example, utilizing reagents such as the MBCAT oligonucleotides, and antibodies directed against an MBCAT, as described above for: (1) the detection of the presence of MBCAT gene mutations, or the detection of either over- or under-expression of MBCAT mRNA relative to the non-disorder state; (2) the detection of either an over- or an under-abundance of  
25 MBCAT gene product relative to the non-disorder state; and (3) the detection of perturbations or abnormalities in the signal transduction pathway mediated by MBCAT.

Thus, in a specific embodiment, the invention is drawn to a method for diagnosing a disease or disorder in a patient that is associated with alterations in MBCAT expression, the method comprising: a) obtaining a biological sample from the patient; b) contacting  
30 the sample with a probe for MBCAT expression; c) comparing results from step (b) with a control; and d) determining whether step (c) indicates a likelihood of the disease or disorder. Preferably, the disease is cancer, most preferably a cancer as shown in TABLE 2. The probe may be either DNA or protein, including an antibody.

## EXAMPLES

The following experimental section and examples are offered by way of illustration and not by way of limitation.

5           I.    C. elegans beta-catenin screen

The identification of mutants that suppress the cell adhesion defect of beta-catenin may lead to unique therapeutic targets that inhibit cell migration or metastasis. *hmp-2* was initially identified in an EMS screen for defects in body elongation during embryonic morphogenesis (see Costa *et al.*, (1998) The Journal of Cell Biology 1998, 141: 297-308).

10   The loss of function allele *hmp-2* (*zu364*) exhibits 99% embryonic lethality, with mutant embryos arresting during elongation and abnormal bulges forming on the dorsal side. About 1% of these embryos hatch to form viable lumpy larvae. The reduction of function allele *hmp-2* (*qm39*) yields viable larvae with a characteristic lumpy appearance. When grown at 15°C, approximately 92% (SD 3.9) of the L1 larvae show this lumpy phenotype,

15   with the penetrance of the phenotype decreasing as the animals molt and move through successive larval stages. For this screen, *hmp-2* (*qm39*) worms were soaked at 15°C in double stranded RNA (dsRNA) at the L4 larval stage and the progeny were scored as L1 larvae for modification of the adhesion defect. The screen protocol is described below.

20           1)    *hmp-2* (*qm39*) animals were bleached and hatched on peptone free agarose plates to produce a synchronous population. Starved L1s were transferred to 10x peptone plates seeded with 750 µl OP50 (25% w/v in TB) and allowed to develop to the L4 larval stage.

              2)    dsRNA was dispensed in 6 µl aliquots into 96 well round bottom plates  
25   (Nunc #262162). L4 animals were collected by suspension in M9 buffer, washed 2x with M9 to remove any excess OP50, and dispensed in 2 µl aliquots into the RNA to a total worm density of 75-100 worms per well. As a control, multiple wells contained only RNA resuspension buffer (1x IM buffer).

              3)    Animals were soaked in dsRNA at 15°C for 24 hours.

30           4)    Following dsRNA soaking, the animals were fed in the wells by addition of 25µl liquid NGM + 3% OP50. The animals were kept at 15°C and allowed to become gravid and lay progeny in the wells, which took approximately 72 hours. Food levels were monitored visually during maturation and more was added as needed.

5) Following maturation, animals from each well were plated onto individual 6cm peptone free agarose plates and placed at 15°C overnight.

6) Animals on each plate were scored visually under the dissecting microscope for modification of the lumpy phenotype. Scoring was performed qualitatively, with an increase in dead embryos scored as enhancement and an increase in wild type appearing animals scored as suppression of the defect.

7) Retests of interesting suppressor candidates followed the same protocol as the primary screen with certain modifications: several retests were performed for each suppressor, retested candidates were encoded so that they could be scored blindly, and retested candidates were scored quantitatively. Each plate was scored by counting 100 total objects. An object was defined as either an embryo or an L1 stage larva. Each object was scored as one of the following: a wildtype appearing animal, a lumpy appearing animal, or an unhatched embryo. Scores were represented as the percentage of wildtype appearing animals relative to all objects scored. Wildtype animals were defined as L1 larvae with smooth cuticles that did not have any sort of lumpy body morphology.

8) A confirmed suppressor was one that was  $\geq 2$  standard deviations away from the mean of the controls for at least 3 of the four retest experiments.

## II. Analysis of Table 1

20 BLAST analysis (Altschul et al., *supra*) was employed to identify Targets from *C. elegans* modifiers. The columns "MBCAT symbol", and "MBCAT name aliases" provide a symbol and the known name abbreviations for the Targets, where available, from Genbank. "MBCAT RefSeq\_NA or GI\_NA", "MBCAT GI\_AA", "MBCAT NAME", and "MBCAT Description" provide the reference DNA sequences for the MBCATs as available from National Center for Biology Information (NCBI), MBCAT protein Genbank identifier number (GI#), MBCAT name, and MBCAT description, all available from Genbank, respectively. The length of each amino acid is in the "MBCAT Protein Length" column.

Names and Protein sequences of *C. elegans* modifiers of beta-catenin from screen (Example I), are represented in the "Modifier Name" and "Modifier GI\_AA" column by GI#, respectively.

TABLE1

BCAT symbol	MBCA T name aliases	MBC AT RefSe q_NA or GI_N A	NA SE Q ID NO	MBCA T GI_AA or RefSeq _AA	AA SEQ ID NO	MBCAT name	MBCAT description	MBC AT protei n lengt h	Modifie r name	Modifier GI_AA
DLG1	hdlg, SAP97	NM_0 04087	1	475816 2 NP_004 078.1	10	gi 4758162 re f NP_004078. 1  discs, large (Drosophila) homolog 1 [Homo sapiens]	Homolog of Drosophila discs large tumor suppressor 1; putative kinase, binds EPB41, may have structural and signaling roles; member of the membrane- associated guanylate kinase (MAGUK) family	926aa	C25F6. 2	13775380
DLG2	PSD- 93, chapsy n-110	NM_0 01364	2	455752 7 NP_001 355.1	11	gi 4557527 re f NP_001355. 1  discs, large (Drosophila) homolog 2 [Homo sapiens]	DLG2 is a member of the membrane- associated guanylate kinase (MAGUK) family. It heteromultimerizes with DLG4. With DLG4 it is recruited into the same NMDA receptor and potassium channel clusters. These 2 MAGUK proteins may interact at postsynaptic sites to form a multimeric scaffold for the clustering of receptors, ion channels, and associated signaling proteins. Chapsyn-110; may mediate organized clustering of NMDA receptors and Shaker-family K+ channel subunits in nerve cells; strongly similar to rat Chapsyn-110	870aa	C25F6. 2	13775380

DLG3	NEDLG, NE-DLG, SAP102	NM_021120	3	10863921 NP_066943.1	12	gi 10863921 ref NP_066943.1  discs, large (Drosophila) homolog 3 [Homo sapiens]	NA	817aa	C25F6.2	13775380
DLG4	PSD95, SAP90, PSD-95	NM_01365	4	4557529 NP_001356.1	13	gi 4557529 ref NP_001356.1  discs, large (Drosophila) homolog 4 [Homo sapiens]	DLG4 is a member of the membrane-associated guanylate kinase (MAGUK) family. It heteromultimerizes with DLG2. With DLG2 it is recruited into the same NMDA receptor and potassium channel clusters. These 2 MAGUK proteins may interact at postsynaptic sites to form a multimeric scaffold for the clustering of receptors, ion channels, and associated signaling proteins.	767aa	C25F6.2	13775380
LOC89363	DLG3	XM_034696	5	14786086 XP_034696.1	14	gi 14786086 ref XP_034696.1  KIAA1232 protein [Homo sapiens]	NA	512aa	C25F6.2	13775380
HSPC111	HSPC185, CGI-117	NM_016391	6	7705451 NP_057475.1	15	gi 7705451 ref NP_057475.1  hypothetical protein; CGI-117 protein [Homo sapiens]	NA	178aa	ZK265.6	17511059
LOC55974	SCP	NM_018845; 8, 9 6563277; 16307087	7, 8, 9	10047124 NP_061333.1	16	gi 10047124 ref NP_061333.1  stromal cell protein [Homo sapiens]	Highly similar to murine Rga; may be a membrane receptor	221aa	K02D7.5	17541198

### III. High-Throughput In Vitro Fluorescence Polarization Assay

Fluorescently-labeled MBCAT peptide/substrate are added to each well of a 96-well microtiter plate, along with a test agent in a test buffer (10 mM HEPES, 10 mM



NaCl, 6 mM magnesium chloride, pH 7.6). Changes in fluorescence polarization, determined by using a Fluorolite FPM-2 Fluorescence Polarization Microtiter System (Dynatech Laboratories, Inc), relative to control values indicates the test compound is a candidate modifier of MBCAT activity.

5

#### IV. High-Throughput In Vitro Binding Assay.

<sup>33</sup>P-labeled MBCAT peptide is added in an assay buffer (100 mM KCl, 20 mM HEPES pH 7.6, 1 mM MgCl<sub>2</sub>, 1% glycerol, 0.5% NP-40, 50 mM beta-mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors) along with a test agent to the wells of a Neutralite-avidin coated assay plate and incubated at 25°C for 1 hour. Biotinylated substrate is then added to each well and incubated for 1 hour. Reactions are stopped by washing with PBS, and counted in a scintillation counter. Test agents that cause a difference in activity relative to control without test agent are identified as candidate beta-catenin modulating agents.

15

#### V. Immunoprecipitations and Immunoblotting

For coprecipitation of transfected proteins,  $3 \times 10^6$  appropriate recombinant cells containing the MBCAT proteins are plated on 10-cm dishes and transfected on the following day with expression constructs. The total amount of DNA is kept constant in each transfection by adding empty vector. After 24 h, cells are collected, washed once with phosphate-buffered saline and lysed for 20 min on ice in 1 ml of lysis buffer containing 50 mM Hepes, pH 7.9, 250 mM NaCl, 20 mM -glycerophosphate, 1 mM sodium orthovanadate, 5 mM p-nitrophenyl phosphate, 2 mM dithiothreitol, protease inhibitors (complete, Roche Molecular Biochemicals), and 1% Nonidet P-40. Cellular debris is removed by centrifugation twice at  $15,000 \times g$  for 15 min. The cell lysate is incubated with 25  $\mu$ l of M2 beads (Sigma) for 2 h at 4 °C with gentle rocking.

After extensive washing with lysis buffer, proteins bound to the beads are solubilized by boiling in SDS sample buffer, fractionated by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membrane and blotted with the indicated antibodies. The reactive bands are visualized with horseradish peroxidase coupled to the appropriate secondary antibodies and the enhanced chemiluminescence (ECL) Western blotting detection system (Amersham Pharmacia Biotech).

30

VI. Kinase assay

A purified or partially purified MBCAT is diluted in a suitable reaction buffer, e.g., 50 mM Hepes, pH 7.5, containing magnesium chloride or manganese chloride (1-20 mM) and a peptide or polypeptide substrate, such as myelin basic protein or casein (1-10  $\mu\text{g/ml}$ ). The final concentration of the kinase is 1-20 nM. The enzyme reaction is conducted in microtiter plates to facilitate optimization of reaction conditions by increasing assay throughput. A 96-well microtiter plate is employed using a final volume 30-100  $\mu\text{l}$ . The reaction is initiated by the addition of  $^{33}\text{P}$ -gamma-ATP (0.5  $\mu\text{Ci/ml}$ ) and incubated for 0.5 to 3 hours at room temperature. Negative controls are provided by the addition of EDTA, which chelates the divalent cation ( $\text{Mg}^{2+}$  or  $\text{Mn}^{2+}$ ) required for enzymatic activity. Following the incubation, the enzyme reaction is quenched using EDTA. Samples of the reaction are transferred to a 96-well glass fiber filter plate (MultiScreen, Millipore). The filters are subsequently washed with phosphate-buffered saline, dilute phosphoric acid (0.5%) or other suitable medium to remove excess radiolabeled ATP. Scintillation cocktail is added to the filter plate and the incorporated radioactivity is quantitated by scintillation counting (Wallac/Perkin Elmer). Activity is defined by the amount of radioactivity detected following subtraction of the negative control reaction value (EDTA quench).

VII. Expression analysis

All cell lines used in the following experiments are NCI (National Cancer Institute) lines, and are available from ATCC (American Type Culture Collection, Manassas, VA 20110-2209). Normal and tumor tissues were obtained from Impath, UC Davis, Clontech, Stratagene, Ardis, Genome Collaborative, and Ambion.

TaqMan analysis was used to assess expression levels of the disclosed genes in various samples.

RNA was extracted from each tissue sample using Qiagen (Valencia, CA) RNeasy kits, following manufacturer's protocols, to a final concentration of 50ng/ $\mu\text{l}$ . Single stranded cDNA was then synthesized by reverse transcribing the RNA samples using random hexamers and 500ng of total RNA per reaction, following protocol 4304965 of Applied Biosystems (Foster City, CA).

Primers for expression analysis using TaqMan assay (Applied Biosystems, Foster City, CA) were prepared according to the TaqMan protocols, and the following criteria: a) primer pairs were designed to span introns to eliminate genomic contamination, and b)

each primer pair produced only one product. Expression analysis was performed using a 7900HT instrument.

Taqman reactions were carried out following manufacturer's protocols, in 25  $\mu$ l total volume for 96-well plates and 10  $\mu$ l total volume for 384-well plates, using 300nM primer and 250 nM probe, and approximately 25ng of cDNA. The standard curve for result analysis was prepared using a universal pool of human cDNA samples, which is a mixture of cDNAs from a wide variety of tissues so that the chance that a target will be present in appreciable amounts is good. The raw data were normalized using 18S rRNA (universally expressed in all tissues and cells).

For each expression analysis, tumor tissue samples were compared with matched normal tissues from the same patient. A gene was considered overexpressed in a tumor when the level of expression of the gene was 2 fold or higher in the tumor compared with its matched normal sample. In cases where normal tissue was not available, a universal pool of cDNA samples was used instead. In these cases, a gene was considered overexpressed in a tumor sample when the difference of expression levels between a tumor sample and the average of all normal samples from the same tissue type was greater than 2 times the standard deviation of all normal samples (i.e.,  $\text{Tumor} - \text{average}(\text{all normal samples}) > 2 \times \text{STDEV}(\text{all normal samples})$  ).

Results are shown in Table 2. Number of pairs of tumor samples and matched normal tissue from the same patient are shown for each tumor type. Percentage of the samples with at least two-fold overexpression for each tumor type is provided. A modulator identified by an assay described herein can be further validated for therapeutic effect by administration to a tumor in which the gene is overexpressed. A decrease in tumor growth confirms therapeutic utility of the modulator. Prior to treating a patient with the modulator, the likelihood that the patient will respond to treatment can be diagnosed by obtaining a tumor sample from the patient, and assaying for expression of the gene targeted by the modulator. The expression data for the gene(s) can also be used as a diagnostic marker for disease progression. The assay can be performed by expression analysis as described above, by antibody directed to the gene target, or by any other available detection method.

Table 2

GI#	SE Q ID NO	Breas t	# of Pai rs	Colo n	# of Pai rs	Head and Neck	# of Pai rs	Kidn ey	# of Pai rs	Lung	# of Pai rs	Ovar y	# of Pai rs	Uteru s	# of Pai rs	Prost ate	# of Pai rs	Skin	# of Pair s	
7705450	6	21%	19	48%	33	13%	8	17%	24	35%	20	55%	11	16%	19	17%	12	67%	3	
1630708	7	9	53%	19	45%	33	50%	8	33%	24	57%	21	83%	12	26%	19	8%	12	67%	3

## WHAT IS CLAIMED IS:

1. A method of identifying a candidate beta-catenin pathway modulating agent, said method comprising the steps of:
  - 5 (a) providing an assay system comprising a MBCAT polypeptide or nucleic acid;
  - (b) contacting the assay system with a test agent under conditions whereby, but for the presence of the test agent, the system provides a reference activity; and
  - (c) detecting a test agent-biased activity of the assay system, wherein a difference between the test agent-biased activity and the reference activity identifies the test agent as
  - 10 a candidate beta-catenin pathway modulating agent.
2. The method of Claim 1 wherein the assay system comprises cultured cells that express the MBCAT polypeptide.
- 15 3. The method of Claim 2 wherein the cultured cells additionally have defective beta-catenin function.
4. The method of Claim 1 wherein the assay system includes a screening assay comprising a MBCAT polypeptide, and the candidate test agent is a small molecule
- 20 modulator.
5. The method of Claim 4 wherein the assay is a binding assay.
6. The method of Claim 1 wherein the assay system is selected from the group consisting
- 25 of an apoptosis assay system, a cell proliferation assay system, an angiogenesis assay system, and a hypoxic induction assay system.
7. The method of Claim 1 wherein the assay system includes a binding assay comprising a MBCAT polypeptide and the candidate test agent is an antibody.
- 30 8. The method of Claim 1 wherein the assay system includes an expression assay comprising a MBCAT nucleic acid and the candidate test agent is a nucleic acid modulator.

9. The method of claim 8 wherein the nucleic acid modulator is an antisense oligomer.
10. The method of Claim 8 wherein the nucleic acid modulator is a PMO.
- 5 11. The method of Claim 1 additionally comprising:  
(d) administering the candidate beta-catenin pathway modulating agent identified in (c) to a model system comprising cells defective in beta-catenin function and, detecting a phenotypic change in the model system that indicates that the beta-catenin function is restored.
- 10 12. The method of Claim 11 wherein the model system is a mouse model with defective beta-catenin function.
13. A method for modulating a beta-catenin pathway of a cell comprising contacting a  
15 cell defective in beta-catenin function with a candidate modulator that specifically binds to a MBCAT polypeptide comprising an amino acid sequence selected from group consisting of SEQ ID NOs:10, 11, 12, 13, 14, 15, and 16, whereby beta-catenin function is restored.
14. The method of claim 13 wherein the candidate modulator is administered to a  
20 vertebrate animal predetermined to have a disease or disorder resulting from a defect in beta-catenin function.
15. The method of Claim 13 wherein the candidate modulator is selected from the group consisting of an antibody and a small molecule.
- 25 16. The method of Claim 1, comprising the additional steps of:  
(d) providing a secondary assay system comprising cultured cells or a non-human animal expressing MBCAT ,  
(e) contacting the secondary assay system with the test agent of (b) or an agent  
30 derived therefrom under conditions whereby, but for the presence of the test agent or agent derived therefrom, the system provides a reference activity; and  
(f) detecting an agent-biased activity of the second assay system,

wherein a difference between the agent-biased activity and the reference activity of the second assay system confirms the test agent or agent derived therefrom as a candidate beta-catenin pathway modulating agent,

5       and wherein the second assay detects an agent-biased change in the beta-catenin pathway.

17. The method of Claim 16 wherein the secondary assay system comprises cultured cells.

10   18. The method of Claim 16 wherein the secondary assay system comprises a non-human animal.

19. The method of Claim 18 wherein the non-human animal mis-expresses a beta-catenin pathway gene.

15   20. A method of modulating beta-catenin pathway in a mammalian cell comprising contacting the cell with an agent that specifically binds a MBCAT polypeptide or nucleic acid.

20   21. The method of Claim 20 wherein the agent is administered to a mammalian animal predetermined to have a pathology associated with the beta-catenin pathway.

22. The method of Claim 20 wherein the agent is a small molecule modulator, a nucleic acid modulator, or an antibody.

25   23. A method for diagnosing a disease in a patient comprising:  
      (a) obtaining a biological sample from the patient;  
      (b) contacting the sample with a probe for MBCAT expression;  
      (c) comparing results from step (b) with a control;  
30       (d) determining whether step (c) indicates a likelihood of disease.

24. The method of claim 23 wherein said disease is cancer.

25. The method according to claim 24, wherein said cancer is a cancer as shown in Table 2 as having >25% expression level.



## SEQUENCE LISTING

<110> Exelixis, Inc.

<120> MBCATs AS MODIFIERS OF THE BETA-CATENIN PATHWAY AND METHODS OF USE

<130> EX02-140C-PC

<150> 60/340,213  
<151> 2001-12-13

<150> 60/340,314  
<151> 2001-12-13

<150> 60/340,322  
<151> 2001-12-13

<150> 60/357,502  
<151> 2002-02-15

<160> 16

<170> PatentIn version 3.1

<210> 1  
<211> 3046  
<212> DNA  
<213> Homo sapiens

<400> 1

gttggaacg gcactgctga gtgagggtga ggggtgtctc ggtatgtgcg ccttggatct	60
ggtgtaggcg aggtcacgcc tctcttcaga cagcccgagc cttcccggcc tggcgcgttt	120
agttcggaac tgcgggacgc cgggtgggcta gggcaagggtg tgtgccctct tcctgattct	180
ggagaaaaat gccggtccg aagcaagata ccagagagc attgcacctt ttggaggaat	240
atcgttcaaa actaagcaa actgaagaca gacagctcag aagttccata gaacgggtta	300
ttaacatatt tcagagcaac ctctttcagg ctttaataga tattcaagaa ttttatgaag	360
tgaccttact ggataatcca aaatgtatag atcgttcaaa gccgtctgaa ccaattcaac	420
ctgtgaatac ttgggagatt tccagccttc caagctctac tgtgacttca gagacactgc	480
caagcagcct tagccctagt gtagagaaat acaggatatca ggatgaagat acacctcctc	540
aagagcatat ttcccacaa atcacaaatg aagtgatagg tccagaattg gttcatgtct	600
cagagaagaa cttatcagag attgagaatg tccatggatt tgtttctcat tctcatattt	660
caccaataaa gccaacagaa gctgttcttc cctctcctcc cactgtccct gtgatccctg	720
tcctgccagt ccctgctgag aatactgtca tcctaccac cataccacag gcaaatacctc	780
ccccagtact ggtcaacaca gatagcttgg aaacaccaac ttacgttaat ggcacagatg	840
cagattatga atatgaagaa atcacacttg aaaggggaaa ttcagggcctt ggtttcagca	900
ttgcaggagg tacggacaac ccacacattg gagatgactc aagtattttc attaccaaaa	960

ttatcacagg gggagcagcc gcccaagatg gaagattgcg ggtcaatgac tgtatattac	1020
aagtaaataga agtagatggt cgtgatgtaa cacatagcaa agcagttgaa gcgttgaaag	1080
aagcaggggtc tattgtacgc ttgtatgtaa aaagaaggaa accagtgtca gaaaaaataa	1140
tggaaataaa gctcattaaa ggtcctaaag gtcttgggtt tagcattgct ggaggtgttg	1200
gaaatcagca tattcctggg gataatagca tctatgtaac caaaataatt gaaggaggtg	1260
cagcacataa ggatggcaaa cttcagattg gagataaact tttagcagtg aataacgtat	1320
gtttagaaga agttactcat gaagaagcag taactgcctt aaagaacaca tctgattttg	1380
tttatttgaa agtggcaaaa cccacaagta tgtatatgaa tgatggctat gcaccacctg	1440
atatcaccaa ctcttcttct cagcctgttg ataaccatgt tagcccatct tccttcttgg	1500
gccagacacc agcatctcca gccagatact cccagtttc taaagcagta cttggagatg	1560
atgaaattac aagggaacct agaaaagttg ttcttcatcg tggctcaacg ggccttgggt	1620
tcaacattgt aggaggagaa gatggagaag gaatatttat ttcttttatc ttagccggag	1680
gacctgctga tctaagtggg gagctcagaa aaggagatcg tattatatcg gtaaacagtg	1740
ttgacctcag agctgctagt catgagcagg cagcagctgc attgaaaaat gctggccagg	1800
ctgtcacaaat tgttgacaaa tatcgacctg aagaatacag tcgttttgaa gctaaaatac	1860
atgattttacg ggagcagatg atgaatagta gtattagttc agggtcaggt tctcttcgaa	1920
ctagccagaa gcgatccctc tatgtcagag ccctttttga ttatgacaag actaaagaca	1980
gtgggcttcc cagtcaggga ctgaacttca aatttggaga tatcctccat gttattaatg	2040
cttctgatga tgaatgggtg caagccaggc aggttacacc agatgggtgag agcgatgagg	2100
tcggagtgat tcccagtaaa cgcagagttg agaagaaaga acgagcccga ttaaaaacag	2160
tgaaattcaa ttctaaaacg agagataaag ggcagtcatt caatgacaag cgtaaaaaga	2220
acctcttttc ccgaaaattc cccttctaca agaacaagga ccagagttag caggaaacaa	2280
gtgatgctga ccagcatgta acttctaatt ccagcgatag tgaaagtagt taccgtggtc	2340
aagaagaata cgtcttatct tatgaaccag tgaatcaaca agaagttaat tatactcgac	2400
cagtgatcat attgggacct atgaaagaca ggataaatga tgacttgatc tcagaatttc	2460
ctgacaaaatt tggatcctgt gttcctcata caactagacc aaaacgagat tatgaggtag	2520
atggaagaga ttatcatttt gtgacttcaa gagagcagat ggaaaaagat atccaggaac	2580
ataaattcat tgaagctggc cagtataaca atcatctata tggaacaagt gttcagttctg	2640
tacgagaagt agcaggaaag ggcaaacact gtatccttga tgtgtctgga aatgccataa	2700
agagattaca gattgcacag ctttacccta tctccatttt tattaaaccc aaatccatgg	2760
aaaatatcat ggaaatgaat aagcgtctaa cagaagaaca agccagaaaa acatttgaga	2820

gagccatgaa actggaacag gagtttactg aacatttcac agctattgta caggggggata 2880  
 cgctggaaga catttacaac caagtgaac agatcataga agaacaatct ggttcttaca 2940  
 tctgggttcc ggcaaaagaa aagctatgaa aactcatgtt tctctgtttc tcttttccac 3000  
 aattccattt tctttggcat ctctttgccc tttcctctgg aaaaaa 3046

<210> 2  
 <211> 3071  
 <212> DNA  
 <213> Homo sapiens

<400> 2  
 aaaagcaact gaggtcttaa ctttcagacg ctgaattctc atctaattga aattactggg 60  
 cataatgcta tatatagcca atgaagagat tttgagctct cactcagtgc cttcaagaca 120  
 tgtcgttttg tagtcagaga aaacagagat caatgcattt tcaaactgac agagggaacg 180  
 gatgctcttt agtagcacat gcccaggatc gtgtgtgtgg ggcttgcgct gtgctgagaa 240  
 gctgaatacc ggtccatag ctccctattt actgcaatgt tctttgcatg ttactgtgca 300  
 ctccggacta acgtgaagaa gtatcgatat caagatgagg acgctccaca tgatcattcc 360  
 ttacctogac taaccacga agtaagaggc ccagaactcg tgcattgtatc agaaaagaac 420  
 ctctctcaaa tagaaaatgt ccatggatat gtccctgcagt ctcatatttc tcctctgaag 480  
 gccagtcttg ctccataat tgtcaacaca gatacttttg acacaattcc ttatgtcaat 540  
 gggacagaaa ttgaatatga atttgaagaa attacactgg agaggggggaa ttctggcctg 600  
 ggattcagta ttgctggggg gacagataat cccacattg gagatgaccc tggcatattt 660  
 attacgaaga ttataccagg aggtgctgca gcagaggatg gcagactcag ggtcaatgat 720  
 tgtatcttgc gggatgaatga ggttgatgtg tcagagggtt cccacagtaa agcgggtggaa 780  
 gccctgaagg aagcagggtc tatcgctcgg ctgtatgtgc gtagaagacg acctattttg 840  
 gagaccgttg tggaaatcaa actgttcaaa ggccctaaag gtttaggctt cagtattgca 900  
 ggaggtgtgg ggaaccaaca cattcctgga gacaacagca tttatgtaac taaaattata 960  
 gatggaggag ctgcacaaaa agatggaagg ttgcaagtag gagatagact actaatggta 1020  
 aacaactaca gtttagaaga agtaacacac gaagaggcag tagcaatatt aaagaacaca 1080  
 tcagaggtag tttattttaa agttggcaac cccactacca tttatatgac tgatccttat 1140  
 ggtccacctg atattactca ctcttattct ccaccaatgg aaaaccatct actctctggc 1200  
 aacaatggca ctttagaata taaaacctcc ctgccacca tctctccagg gaggtactca 1260  
 ccaattccaa agcacatgct tgttgacgac gactacacca ggccctccga acctgtttac 1320  
 agcactgtga acaaactatg tgataagcct gcttctccca ggcactattc cctgttgag 1380

```

tgtgacaaaa gcttcctcct ctcagctccc tattcccact accacctagg cctgctacct 1440
gactctgaga tgaccagtca ttcccaacat agcaccgcaa ctcgtcagcc ttcaatgact 1500
ctccaacggg ccgtctccct ggaaggagag cctcgcaagg tagtcctgca caaaggctcc 1560
actggcctgg gcttcaacat tgtcgggtgg gaagatggag aaggatattt tgtgtccttc 1620
attctggctg gtggaccagc agacctaatg ggggagctcc agagaggaga ccagatccta 1680
tcggtgaatg gcattgacct ccgtgggtgca tcccacgagc aggcagctgc tgcactaaag 1740
ggggctggac agacagtgc gattatagca caatatcaac ctgaagatta cgctcgattt 1800
gaggccaaaa tccatgacct acgagagcag atgatgaacc acagcatgag ctccgggtcc 1860
ggatccctgc gaaccaatca gaaacgctcc ctctacgtca gagccatgtt cgactacgac 1920
aagagcaagg acagtgggct gccaaagtcaa ggacttagtt ttaaatatgg agatattctc 1980
cacgttatca atgcctctga tgatgagtgg tggcaagcca ggagagtcac gctggagggg 2040
gacagtgagg agatgggggt catccccagc aaaaggaggg tggaaagaaa ggaacgtgcc 2100
cgattgaaga cagtgaagtt taatgccaaa cctggagtga ttgattcgaa agggctcattc 2160
aatgacaagc gtaaaaagag cttcatcttt tcacgaaaat tccattcta caagaacaag 2220
gagcagagtg agcaggaaac cagtgatcct gaacgtggac aagaagacct cattctttcc 2280
tatgagcctg ttacaaggca ggaaataaac tacaccggc cggtgattat cctggggccc 2340
atgaaggatc ggatcaatga cgacttgata tctgaattcc ctgataaatt tggctcctgt 2400
gtgcctcata ctacgaggcc aaagcgagac tacgaggtgg atggcagaga ctatcacttt 2460
gtcatttcca gagaacaaat ggagaaagat atccaagagc acaagtttat agaagccggc 2520
cagtacaatg acaatttata tggaaccagt gtgcagtctg tgagatttgt agcagaaaga 2580
ggcaaacact gtatacttga tgtatcagga aatgctatca agcggttaca agttgcccag 2640
ctctatccca ttgccatctt cataaaaccc aggtctctgg aatctcttat ggagatgaat 2700
aagcgtctaa cagaggaaca agccaagaaa acctatgatc gagcaattaa gctagaacaa 2760
gaatttggag aatattttac agctattgtc caaggagata ctttagaaga tatatataac 2820
caatgcaagc ttgttattga agagcaatct gggcctttca tctggattcc ctcaaaggaa 2880
aagttataaa ttagctactg cgcctctgac aacgacagaa gagcatttag aagaacaaaa 2940
tatatataac atactacttg gaggtcttta tgtttttgtt gcatttatgt ttttgcagtc 3000
aatgtgaatt cttacgaatg tacaacacaa actgtatgaa gccatgaagg aaacagaggg 3060
gccaaagggg g 3071

```

<210> 3  
 <211> 3100  
 <212> DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 3

tgctggaggg ggagccgtgg gtgcgggggca ccgtggggggc cgaggcccccg ggacgcccgc	60
ccggccccag gccccgctca gcccgggcgc ccccacgggt gcccccccct tcttggtccg	120
agcagtgtga gtgtgccagg gagcccgccg gcggcgggcg cggtggtggc ggcgggtggc	180
gcggcgtgga atccggcgtg ggctgggggg tccgagccgc ggggggcagt gccatgcaca	240
agcaccagca ctgctgtaag tgccctgagt gctatgagggt gaccgcctg gccgcctgc	300
ggcgccctga gcctccgggg tacggcgact ggcaagtccc cgacccttac gggccagggtg	360
ggggcaacgg cgccagcgcg ggttatgggg gctacagctc gcagaccttg ccctcgagg	420
cgggggccac cccaccccct cgcaccaagg ccaagctcat cccacccggc cgggatgtgg	480
ggccgggtgcc tcttaagcca gtcccgggca agagcaccoc caaactcaac ggcagcggcc	540
ccagctggtg gccagagtgc acctgtacca accgggactg gtatgagcag gtgaatggca	600
gtgatggcat gttcaaatat gaggaatcg tacttgagag gggcaactct ggcctgggct	660
tcagtatcgc aggtggcatc gacaatcccc atgtccctga tgaccctggc atttttatta	720
ccaagattat ccctgggtgga gcagctgcca tggatgggag gctgggggtg aatgactgtg	780
tgctgccggg gaatgagggt gaagtgtcgg aggtggtaca cagccggggc gtggaggcgc	840
tgaaagaggc aggcctctgt gtgcgattgg tgggtgcggag ggcacagcct ccacccgaga	900
ccatcatgga ggtcaacctg ctcaaagggc ccaaaggcct gggtttcagc attgctgggg	960
gtattggcaa ccagcacatc ccaggagaca acagcatcta catcaccaag atcattgagg	1020
ggggtgctgc tcagaaggat ggacgcctac agattggggga ccggtgctg gcggtgaaca	1080
acaccaatct gcaggatgtg aggcacgagg aagctgtggc ctcaactgaag aacacatctg	1140
atatggtgta tttgaagggt gccaaagccag gcagcctcca cctcaacgac atgtacgctc	1200
cccctgacta cgccagcact tttactgcct tggctgacaa ccacataagc cataattcca	1260
gcctgggtta tctcggggct gtggagagca aggtcagcta ccctgctcct cctcagggtc	1320
ccccacccg ctactctcct attcccaggc acatgctggc tgaggaggac ttcaccagag	1380
agcctcgcaa gatcatcctg cacaaaggct ccacaggcct gggcttcaac atcgtaggag	1440
gagaggatgg agaaggcatt tttgtctcct tcatcctggc aggaggccca gctgacctga	1500
gtggggagct gcgcagggga gaccggatct tatcggtgaa tggagtgaat ctgagggaatg	1560
caactcatga gcaggctgca gctgctctga aacggggccgg ccagtcagtc accattgtgg	1620
cccagtacag acctgaagaa tacagtcgct ttgaatcgaa gatacatgac ttacgagaac	1680
aaatgatgaa cagcagcatg agctctgggt ctgggtccct ccgaacaagt gaaaagagggt	1740
ccttgatgtg cagggccctg tttgattatg atcggactcg ggacagctgc ctgccaaagcc	1800

```

aggggctcag cttctcttat ggtgacattc tgcatgtcat taatgcctct gatgatgagt 1860
ggtggcaggc aaggctggtg accccacacg gagaaagtga gcagatcggg gtgatcccca 1920
gtaagaagag ggtggaaaag aaagaaagag ctcgattgaa aactgtgaag ttccatgcca 1980
ggacggggat gattgagtct aacagggact tcccgggggt aagtgcgat tattatggag 2040
caaagaacct gaaaggacaa gaggatgcta ttttgtcata tgagccagt acacggcaag 2100
aaattcacta tgcaaggcct gtgatcatcc tgggcccacat gaaggaccga gtcaatgatg 2160
acctgatctc cgaatttcca cataaatttg gatcctgtgt gccacatact acccggcctc 2220
gacgtgataa tgaggtggat ggacaagact accactttgt ggtgtcccg gaacaaatgg 2280
agaaagatat tcaggacaac aagttcatcg aggcggggcca atttaatgat aacctctatg 2340
ggaccagcat ccagtcagt cgggcagttg cagagagggg caagcactgc atcttagatg 2400
tttccggcaa tgctatcaag agactgcagc aagcacaact ttacccatt gccattttca 2460
tcaagcccaa gtccattgaa gcccttatgg aaatgaaccg aaggcagaca tatgaacaag 2520
caaataagat ctatgacaaa gccatgaaac tggagcagga atttggagag tactttacag 2580
ccattgtaca ggggtgactca ctggaagaga ttataacaa aatcaaaca atcattgagg 2640
accagtctgg gcactacatt tgggtcccat cccctgaaaa actctgaaga atccctcca 2700
accattctct tgtgaacaga agaaatcaag tccctcttcc ctccctccctc ttcatctctg 2760
tcccatggg gagaacaaat gctactgttc ttgtccctt ttttagatat gtcaaaaaa 2820
attaagtttt ctagtctgt tcttttttt ttttaagtt ttgtttgtt cagtttattt 2880
tttgggatga tgccatctca ttcacatgt gactgtgcc attcctgcat ggaccttcc 2940
caagcgctag cataggtgca aaatccatca gagccattgt tttcataaaa accaagcaga 3000
agtgaagaga aaagaggagg actgatggaa agacagactc tggacagctg cacggcttgt 3060
gaagtgagct aaatgcacca catgatgaga tgctcctggg 3100

```

```

<210> 4
<211> 3995
<212> DNA
<213> Homo sapiens

```

```

<400> 4
ggatccgcgg gacagatgag gaaggggctt aagtcactgc agccagaggg atggagggtg 60
actgatggga gggcttctcc ggtgggggtta gaagggaaaa gtagggaaag agaagtgtaa 120
ggtagatggc agaggcagag acatggaaag acagactcta gggttcctga tgatatctat 180
ctcggccaac aaaaaggga gggtagagt gtgggggcac ccaagctagg gtgtgagtac 240
cctaagtgtt ttcttctgag atgtaggcca ttcactaact cttggaacag ctacagtttc 300

```

acagtaggaa gaccccccca gattcactgc ccctccctta gtaaagcctc tgagaccttc	360
ctgaacattc ctttctgtct ttgccctctg ttccctccag agactatgtg cccaggcaga	420
tggattccctc ccgggcctga gaggaactgc aggaattctc ctgcctctta cccgtaaaac	480
cccaacttct ctagccctag ggcaggaagt cccaaacaat ttctaccctt ttttctgcaa	540
ttctcattgg ggtgagagga ggcccaggag gagagagagc tgggctcagc ttctttttga	600
gctgctggag ccctctgtga ggaggccctc tttgctggct tctcaggaga gtgtggctag	660
gttctgcctg cctatgggaa gagggggcca gggtgtgtgg agcaagatgg tgcggtgctg	720
gtgccttggg acctggggga atgggacagc tggtcggctc agagacggcc tactttactc	780
acagctggaa tttagtgggg agaagcagct caactccaat cctggaggat tagggagatt	840
aaagtgagag aagagagaga tgtcccagag accaagagct cccaggctag ccctctggct	900
cctggcaccc ccactgctgc ggtgggcacc cccactcctc acagtgcctgc atagcgacct	960
cttccaggcc ttgctggaca tcctggacta ttatgaggct tccctctcag agagtcagaa	1020
ataccgcta ccaagatgaag acacgcccc tctggagcac agcccgggcc acctcccaa	1080
ccaggccaat tctccccag tgattgtcaa cacagatacc ctagaagccc caggatatga	1140
gttgacagtg aacgggaccg agggggagat ggaatacag gaaatcacat tggaaagggg	1200
taactcaggt ctgggcttca gcatcgagc tggcactgac aaccacaca tgggtgacga	1260
cccatccatt ttcatcacca agatcattcc tgggtgggct gcggcccagg atggccgcct	1320
cagggtcaac gacagcatcc tgtttgtaaa tgaagtggac gtgcgcgagg tgaccactc	1380
agcggcggtg gaagccctca aagaggcagg ctccatcgct cgccctctatg tcatgcgcg	1440
gaagcccccg gctgagaagg tcatggagat caagctcatc aaggggccta aaggctcttg	1500
cttcagcatc gcagggggcg tagggaaacca gcacatccca ggagataata gcatctatgt	1560
aacaaagatc atcgaagggg gtgctgcca caaggatggg aggttgacaga ttggagacaa	1620
gaccttggtg gtcaacagtg tggggctaga ggacgtcatg catgaagatg ctgtggcagc	1680
cctgaagaac acgtatgatg ttgtctacct aaagggtggc aagcccagca atgcctacct	1740
gagtgcagc tatgtcccc cagacatcac aacctcttat tcccagcacc tggacaatga	1800
gatcagtcac agcagctacc tgggcaccga ctacccaca gccatgaccc ccacttcccc	1860
tgggcgctac tctccagtgg ccaaggacct gctcggggag gaagacattc cccgagaacc	1920
gaggcgaatt gtgatccacc ggggctccac gggcctgggc ttcaacatcg tgggtggcga	1980
ggacggtgaa ggcattctca tctcctttat cctggccggg ggccttgagc acctcagtgg	2040
ggagctgcgg aagggggacc agatcctgtc ggtcaacggg gtggacctcc gaaatgccag	2100
ccatgagcag gctgccattg ccctgaagaa tgcgggtcag acggtcacga tcatcgctca	2160

gtataaacca gaagagtaca gccgattcga ggccaagatc caogaccttc gggaacagct	2220
catgaacagc agcctgggct cagggactgc gtccttgctg agcaacccca aaaggggttt	2280
ctacatcagg gccctgtttg attacgacaa gaccaaggac tgcggcttcc tgagccaggg	2340
cctgagcttc cgctttgggg atgtgctgca tgtcatcgat gctagtgatg aggagtgggtg	2400
gcaggcacgg cggggtccact ctgacagtga gaccgacgac attgggttca tccccagcaa	2460
acggcggggt gagcgacgag agtgggtcaag gttaaaggcc aaggactggg gctccagctc	2520
tggatcgag ggtcgagaag actcggttct gagctacgag acagtgacgc agatggaagt	2580
gcactatgct cgccccatca tcctccttgg gccaccaag gaccgcgcca acgatgatct	2640
tctctccgag tttcccgaca agtttggatc ctgtgttccc catacgacac ggcccaagcg	2700
ggagtatgag atagatggcc gggattacca ctttgtgtcg tcccgggaga aaatggagaa	2760
ggacattcag gcgcacaagt tcattgaggc cggccagtac aacagccacc tctatgggac	2820
cagcgtccag tccgtgcgag aggtggcaga gcaggggaag cactgcatcc tcgatgtctc	2880
ggccaatgcc gtgcggcggc tgcaggcggc ccacctgcac cccatcgcca tcttcatccg	2940
cccccgctcc ctggagaatg tgctagagat taacaagcgg atcacagagg agcaagcccg	3000
caaagccttc gacagagcca ccaagctgga gcaggagtcc acagagtgct tctcagccat	3060
cgtggaggggt gacagctttg aggagatcta ccacaagggtg aagcgtgtca tcgaggacct	3120
ctcaggcccc tacatctggg ttccagcccc agagagactc tgattcctgc cctggcttgg	3180
cctggactcg ccctgcctcc atcacctggg cccttggctc ggactgaatt gcccaagccc	3240
ttggctcccc cgggcctccc tcccacccct tcttatttat ttcctttcta actggatcca	3300
gcctgttggg ggggggacac tcctctgcat gtatccccgc accccagaac tgggctcctg	3360
aacgccagga acctggggtc tgggggggag ctgggctcct tgttccgagc ccttgctcct	3420
taggatcccc gccccacct gcccccaatg cacacacaga cccaccgggg gccacctgcc	3480
ctcccccatc ctctcccaca cacattccag aagtcagggc cccctcgagg agcaccgct	3540
gcagggatgc agggccacag gcctccgctc tctcctaagg cagggtctgg ggtcacccct	3600
gcctcatcgt aattccccat gttaccttga tttctcattt attttttcca ctttttttct	3660
tctcaaagggt ggttttttgg ggggagaagc aggggactcc gcagcgggcc cctgccttcc	3720
acatgcccc accatttttc tttgccggtt tgcattgagt gaaggctctaa atgtggcttt	3780
tttttttttt ttcctgggaa ttttttggg gaaaaggag ggatgggtct agggagtggg	3840
aaatgcggga gggagggtgg ggcagggtc gggggctggg tgtccgggag ccagggaaga	3900
ctggaaatgc tgccgccttc tgcaatttat ttattttttt cttttgagag agtgaaagga	3960
agagacagat acttgaaaaa aaaaaaaaaa aaaaa	3995



<210> 5  
 <211> 5073  
 <212> DNA  
 <213> Homo sapiens

<400> 5  
 gggctgagga ggcgagggca gcggagtttc ctgccctgag atagttgcag cccgctctgc 60  
 tgcaaccatt tcaaattaga gaagaaagca gttcagccgg tggggctggc gggactctga 120  
 cgggcccggt ggcctcgccg gcaacggccc cgccccgctg gcggcagcgg cggcggcagc 180  
 ggctgcgcgg ggcctggaac tggtcgggcc ggctgggggt cggggggaca ggtttgagg 240  
 gtggggcccg agaggctggc gggcaggcag cagtggagcc ggaagggtag gcagccaggg 300  
 gagagagaga ggagctatgg agagggcccg caagttctcg ggctccggct tggccatggg 360  
 cttgggctcc gcctccgctt cggcctggag gagggtctcg cagaggtggg cctggccgct 420  
 ccgctccctg cggcccgag gggatgccag agagcctcgc aagatcatcc tgcacaaagg 480  
 ctccacaggc ctgggcttca acatcgtagg aggagaggat ggagaaggca tttttgtctc 540  
 cttcatcctg gcaggaggcc cagctgacct gagtggggag ctgcccaggg gagaccggat 600  
 cttatcggtg aatggagtga atctgaggaa tgcaactcat gaggaggctg cagctgctct 660  
 gaaacggggc ggccagtcag tcaccattgt ggcccagtac agacctgaag aatacagtcg 720  
 ctttgaatcg aagatacatg acttacgaga acaaatgatg aacagcagca tgagctctgg 780  
 gtctgggtcc ctccgaacaa gtgaaaagag gtccttgatg gtcagggccc tgtttgatta 840  
 tgatcggact cgggacagct gcctgccaag ccaggggctc agcttctctt atggtgacat 900  
 tctgcatgtc attaatgcct ctgatgatga gtgggtggcag gcaaggctgg tgacccaca 960  
 cggagaaaagt gaggagatcg gtgtgatccc cagtaagaag aggggtggaaa agaaagaaag 1020  
 agctcgattg aaaactgtga agttccatgc caggacgggg atgattgagt ctaacaggtc 1080  
 gatcaaaacg aaacgtaaaa agagtttccg cctctctcga aagtttccat ttacaagag 1140  
 caaagaaaac atggcccagg agagcagcat acaggaacag ggagtgcacat ccaacaccag 1200  
 tgacagcgaa agcagttcca aaggacaaga ggatgctatt ttgtcatatg agccagtgac 1260  
 acggcaagaa attcactatg caaggcctgt gatcatcctg ggcccaatga aggaccgagt 1320  
 caatgatgac ctgatctccg aatttccaca taaatttgga tcctgtgtgc cacatactac 1380  
 ccggcctcga cgtgataatg aggtggatgg acaagactac cactttgtgg tgtcccgaga 1440  
 acaaatggag aaagatattc aggacaacaa gttcatcgag gcgggccaat ttaatgataa 1500  
 cctctatggg accagcatcc agtcagtgcg ggcagttgca gagaggggca agcactgcat 1560  
 cttagatggt tccggcaatg ctatcaagag actgcagcaa gcacaacttt accccattgc 1620  
 cattttcatc aagcccaagt ccattgaagc ccttatggaa atgaaccgaa ggcagacata 1680

tgaacaagca aataagatct atgacaaagc catgaaactg gagcaggaat ttggagagta	1740
ctttacagcc attgtacagg gtgactcact ggaagagatt tataacaaaa tcaaacaaat	1800
cattgaggac cagtctgggc actacatttg ggtcccatcc cctgaaaaac tctgaagaat	1860
ccccccaac cattctcttg tgaacagaag aaatcaagtc cctcttccct cctccctctt	1920
cattcctgtc cccatgggga gaacaaatgc tactgttctt gtcccctttt ttagatatgt	1980
caaaaaaaaaat taagttttct agtcctgttc tttttttttt ttttaagttt tgtttgtttc	2040
agttttatttt ttgggatgat gccatctcat tcatcatgtg actgtgcca ttcctgcatg	2100
gacctttccc aagcgctagc acagggtgca aatccatcag agccattgtt ttcataaaaa	2160
ccaagcagaa gtgaagagaa aagaggagga ctgatggaaa gacagactct ggacagctgc	2220
acggcttggtg aagtgtgcta aatgcaccac atgatgagat gctcctgggc atttctccct	2280
atctgtactg ctgtcttgca gctctgaacg gtgcaacgta atggcgacag aaagtatctt	2340
atztatatat agatatatat atgtaattta tataaaatat atagaaatta ttatatatat	2400
atattataca ctctcatata atatatatat attcacacac atttgggtta gaaaatctat	2460
ggagacttca tcaatggtac tatgttatta gagaaatgct ttaattttca tattccaatc	2520
agatggcatc ttctatccca gctggttggg aagggttga agatggtagc aagtgtgca	2580
ctaagggcac tcgcatttgg tcattctctt gagagctagg aggggtcagc ctgaggccgg	2640
agaggaaggg cttttgcctg ggggtgagag gtgagagact tgacctgaaa gcagctgctg	2700
cccctgaggt gcagccagag tcctgtgtgc ggacagagag aatggctaata gatgctccgc	2760
gtggaattgc ctattgtttt atgccacctg atgtgtctgc atgagagggt tcctttttgt	2820
tccttttttaa gctgctgtta aaccaaacc atgttgtgt actgtgtcag ctttttcatt	2880
attgcaaatt ttacttttac tatttaagt aatgcataga atccaatttg ccaagggtct	2940
aaaggcttat gaggtcctga aggagccagg ccttgtgatg gagtaggtga cacaggcctg	3000
gttgtcctgt cagcagaagg gaaagcaggg gctgggctga gaggaggaca cggagggctc	3060
tgctgaggtt ccttcctggg ttccaccaac agggacaggg agtcacttgc cttccagttc	3120
tgtgtcggga tggcgggaca gcacttggt tgcttgcca gctgcgtcat gagtttgatt	3180
tggttttttt ttttttgag ctgcttcata tgctctgtc cagccctcc ccaacagctg	3240
gtagcttatg gtttcttcaa gaggaagta gactttatgc tgtacatttg agctgtagag	3300
ctaagattcg cttactggtg agctgtgaaa ccttgttgct ttttccaga gtctgatggc	3360
agtgtactgt atcaagggaa tcttcaccgc cacaagtgc ggcagcaggt gtggttcagg	3420
tccccccca cccactgtg ctcttttgaa gccaacgtgc ctccctcgcc tccatactgg	3480
agggacgacg caggggagaa cagagaagtg cttggcccta ggattgaggc acttgtttcc	3540

tagcccgctg ggttagggct ggtgcaagcg aggcaatgtt gaggatgctt taagcactac 3600  
 cagccgaatc cggaactct gttaacagtt gtccaaccag cagaatgagg ctaactgtat 3660  
 aaagcatggg acccaggatg aggataagga aaggacagcg gctttccctg ggcagtacaa 3720  
 tggcttgaag gcaaaaaggg ataaagtac agccgactgt gactctggtg aggaggggtg 3780  
 agcagggagg ttgattctct gatgttaact aagtggcaaa gtctcaaccg tgctcagccc 3840  
 tccccctccc aggaagaga aacaaagatt caaagtaagc atgatactag tgggtttacc 3900  
 agtgtttctt ccaaggagac atatatTTTT taataaacga tagttgcaat gaactgtggc 3960  
 tcagagacct tcttaaagta gttgagaagg gagggcggtg gcaaagcagt ggaagaaca 4020  
 tcccaaactt ttgggggcca gagggctctc tccttagtga tgatcagcta gccgagctgg 4080  
 gccgtcctgg ggatcggtag agtccctgg ggtggtgaca ggccctttgt gaaagtgtg 4140  
 tgcttggtct tccacccag cccagacac tgcttcaa atgcaccaacc agatgggagt 4200  
 ccacatctgt ggtggcaaaa tgctgacatt tcccaagag gtacacaagg tgggagaggc 4260  
 ctgctgtagc agaggtgtgt gttagagaaa gcaggggcct gatttagtag cagagaactg 4320  
 ggtgagaaaa atggccagag aaagtgcct gccagctacc agtgtttccg aaaatgaggg 4380  
 tgggatgggc ccatttgcag agcaggacag cagtcatccc catagccctc tgaggagggg 4440  
 agggatgctt agagcaggca gttctggcag ttctgacgtg gcaggtgcca ttgcaacttg 4500  
 tgccggaggag tcttaggaag tgctgtcata attcataagg tcaagagcaa catctggatg 4560  
 aatgagccac ctgaaatgtg tgtgggctga gccacaggaa gggtgagtcc tcttgcttgt 4620  
 ggtgctttat ggtgtgcagg ttgcttgctt tccacattc tctcatttgc ttgaaggcag 4680  
 cctaacaaaa gggagtcccc aaagagctcc atgagagctt aagaaaattc atctcctgag 4740  
 gacaaagaga acaaagatct gtctgtggt cactgttg aggctctgtc ttcacagctg 4800  
 atgttctcct agtggaggta cagctgaaga acattcagcc ccagcacgag aagatacaga 4860  
 gcaacttga aaccccaaag ggagacacac ccttaacact gccgtgctgt gctgtgccgt 4920  
 gtctgaagg aggagagagc cctatctcct cctggttttg ttgctgacat tgcagctgat 4980  
 catgccttga ctcttcatc tctaacagag gaagaactgt attatcaaac cttatgggtc 5040  
 acccatgaat aaataaaata ttgtttcaag cac 5073

<210> 6  
 <211> 910  
 <212> DNA  
 <213> Homo sapiens

<400> 6  
 gttacacgag gtctgagaga cagaggcagc gtgtttgagc tgctgggtgcg gtggtcagcg 60

```

cgatgccc aa ggccaagggc aaaacccgga ggcagaagtt tggttacagt gtcaaccgaa 120
agcgtctgaa ccggaatgct cgacggaagg cagcgcgcg gacgaatgc tcccacatcc 180
gacatgcctg ggaccacgct aaatcggtac ggcagaacct ggccgagatg gggttggctg 240
tggaccccaa cagggcggtg cccctccgta agagaaaggt gaaggccatg gaggtggaca 300
tagaggagag gcctaaagag cttgtacgga agccctatgt gctgaatgac ctggaggcag 360
aagccagcct tccagaaaag aaaggaaata ctctgtctcg ggacctcatt gactatgtac 420
gctacatggt agagaaccac ggggaggact ataaggccat ggcccgtgat gagaagaatt 480
actatcaaga tacc caaaa cagattcgga gtaagatcaa cgtctataaa cgcttttacc 540
cagcagagt gcaagacttc ctcgattctt tgcagaagag gaagatggag gtggagt gac 600
tggtttacat cacagctgcc ccaggctgag gcgtcccccg gaccagt gaa gctggagcca 660
gggtgtaagg caaggaggtg ctgtgtggct ccagaggagc tggccaggtc ccatggaatc 720
agaaggttac acacacacgt gcacactccc cgctctgggg aaggaactgt tctcagaggc 780
tccaatttat attcatctgg gggttcacgg aaaagccaga acctgctgtt ttcagggtgg 840
gtgatgtaaa tatagtgtgt acataataaa gcaaatatat tttaaaaaaaa aaaaaaaaaa 900
aaaaaaaaa 910

```

```

<210> 7
<211> 1316
<212> DNA
<213> Homo sapiens

```

```

<400> 7
ggtaggtccc ggcaaccgca gctcgcgggc ggcgctgggc gggatccgac tctagtcgta 60
atggaggcgg gcggctttct ggactcgctc atttacggag catgcgtggg ctccaccctt 120
ggcatgttct ccgccggcct ctcggaacct aggcacatgc gaatgacctg gagtgtggac 180
aacgtcagat tcttgccctt tctcaccacg gaagtcaaca acctgggctg gctgagttat 240
ggggctttga agggagacgg gatcctcatc gtcgtcaaca cagtgggtgc tgcgcttcag 300
accctgtata tcttggcata tctgcattac tgccctcgga agcgtgttgt gtccttacag 360
actgcaacct tgctaggggt ccttctcctg gggtatggct acttttggct cctggtacct 420
aacctgagg cccggcttca gcagttgggc ctcttctgca gtgtcttcac catcagcatg 480
tacctctcac cactggctga cttggctaag gtgattcaaa ctaaatcaac ccaatgtctc 540
tctaccacac tcaccattgc tacccttctc acctctgcct cctggtgcct ctatgggttt 600
cgactcagag atccctatat catggtgtcc aactttccag gaatcgtcac cagctttatc 660
cgetctggc ttttctggaa gtacccccag gagcaagaca ggaactactg gtcctgcaa 720
acctgaggct gctcatctga ccactgggca ccttagtgcc aacctgaacc aaagagacct 780

```

ccttgttttca gctgggcctg ctgtccagct tcccagggtgc agtggggtgt gggaacaaga 840  
 gatgacttttg aggataaaaag gaccaaagaa aaagctttac ttagatgatt gattgggggcc 900  
 taggagatga aatcactttt tatttttttag agattttttt ttttaatttt ggagggttggg 960  
 gtgcaatctt tagaatatgc cttaaaaggc cgggcgcggt ggctcacgcc tgtaatccca 1020  
 gcacttttggg aggccaaggt gggcggatcg cctgagggtca ggagttcaag accaacctga 1080  
 ctaacatggt gaaaccccat ctctactaaa aatacaaaat tagccaggca tgatggcaca 1140  
 tgcctgtaat ccagataact tgggaggctg aggcaggaga attgcttgaa ccaggagggt 1200  
 ggagggttga gtgagctgag atcgtgccat tgtgatatga atatgcctta tatgctgata 1260  
 tgaatatgcc ttaaaataaa gtgttcccca cccctgaaaa aaaaaaaaaa aaaaaa 1316

<210> 8  
 <211> 1183  
 <212> DNA  
 <213> Homo sapiens

<400> 8  
 cggcaaccgc agctcgccgc gggcgctggg cgggatccga ctctagtcgt aatggaggcg 60  
 ggcggtcttc tggactcgct catttacgga gcatgcgtgg tcttcacct tggcatgttc 120  
 tccgccggcc tctcgacact caggcacatg cgaatgacct ggagtgtgga caacgtcagt 180  
 cctgcccttt ctcaaccaagg aagtcaacgt gttgtgtctc tacagactgc aacctgcta 240  
 ggggtccttc tcctgggtta tggctacttt tggctcctgg taccacaacc tgaggccccg 300  
 cttcagcagt tgggcctctt ctgcagtgtc ttcaccatca gcatgtacct ctcaacctg 360  
 gctgacttgg ctaagggtgat tcaaactaaa tcaacccaat gtctctccta cccactcacc 420  
 attgctaccc ttctcacctc tgccctcctg tgccctctatg ggtttcgact cagagatccc 480  
 tataatcatgg tgtccaactt tccaggaatc gtcaccagct ttatccgctt ctggcttttc 540  
 tggaagtacc ccaggagca agacaggaac tactggctcc tgcaaacctg aggctgctca 600  
 tctgaccact gggcacctta gtgccaacct gaaccaaaga gacctccttg tttcagctgg 660  
 gcctgctgtc cagcttccca ggtgcagtgg gttgtgggaa caagagatga ctttgaggat 720  
 aaaaggacca aagaaaaagc tttacttaga tgattgattg gggcctagga gatgaaatca 780  
 ctttttattt tttagagatt ttttttttta attttgagg ttggggtgca atcttttagaa 840  
 tatgccttaa aaggccgggc gcggtggctc acgcctgtaa tcccagcact ttgggaggcc 900  
 aagggtggcg gatcgctga ggtcaggagt tcaagaccaa cctgactaac atggtgaaac 960  
 cccatctcta ctaaaaatac aaaattagcc aggcattgat gcacatgcct gtaatccag 1020  
 atacttggga ggctgaggca ggagaattgc ttgaaccag gaggtggagg ttgcagtga 1080

ctgagatcgt gccattgtga tatgaatatg ccttatatgc tgatatgaat atgccttaaa 1140  
 ataaagtgtt cccaccccct gaaaaaaaaa aaaaaaaaaa aaa 1183

<210> 9  
 <211> 1186  
 <212> DNA  
 <213> Homo sapiens

<400> 9  
 gtaggtcccg gcaaccgcag gctcgcggcg ggcgctgggc gcgggatccg actctagtcg 60  
 taatggaggc gggcggcttt ctggactcgc tcatttacgg agcatgcgtg gtcttcaccc 120  
 ttggcatgtt ctccgccggc ctctcggacc tcaggcacat gcgaatgacc cggagtgtgg 180  
 acaacgtcca gttcctgccc tttctcacca cggaagtcaa caacctgggc tggctgagtt 240  
 atggggcttt gaaggagac gggatcctca tcgtcgtcaa cacagtgggt gctgcgcttc 300  
 agaccctgta tatcttgga tatctgcatt actgccctcg gaaggctaag gtgattcaaa 360  
 ctaaataaac ccaatgtctc tcctaccac tcaccattgc tacccttctc acctctgcct 420  
 cctggtgcct ctatgggttt cgactcagag atccctatat catgggtgtcc aactttccag 480  
 gaatcgtcac cagctttatc cgcttctggc ttttctggaa gtacccccag gagcaagaca 540  
 ggaactactg gctcctgcaa acctgaggct gctcatctga ccactgggca ccttagtgcc 600  
 aacctgaacc aaagagacct ccttgtttca gctgggcctg ctgtccagct tcccagggtg 660  
 agtgggttgt gggaacaaga gatgactttg aggataaaag gaccaaagaa aaagctttac 720  
 ttagatgatt gattggggcc taggagatga aatcactttt tatttttttag agattttttt 780  
 ttttaatttt ggaggttggg gtgcaatctt tagaatatgc cttaaaaggc cgggcgcggg 840  
 ggctcacgcc tgtaatccca gcactttggg aggccaaggt gggcggatcg cctgaggtca 900  
 ggagttcaag accaactga ctaacatggt gaaaccccat ctctactaaa aatacaaaat 960  
 tagccaggca tgatggcaca tgcctgtaat ccagataact tgggaggctg aggcaggaga 1020  
 attgcttgaa ccaggagggt ggaggttgca gtgagctgag atcgtgccat tgtgatatga 1080  
 atatgcctta tatgctgata tgaatatgcc ttaaaataaa gtgttcccca cccctgcaaa 1140  
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaa 1186

<210> 10  
 <211> 926  
 <212> PRT  
 <213> Homo sapiens

<400> 10

Met Pro Val Arg Lys Gln Asp Thr Gln Arg Ala Leu His Leu Leu Glu  
 1 5 10 15

Glu Tyr Arg Ser Lys Leu Ser Gln Thr Glu Asp Arg Gln Leu Arg Ser  
                   20                                  25                                  30

Ser Ile Glu Arg Val Ile Asn Ile Phe Gln Ser Asn Leu Phe Gln Ala  
                   35                                  40                                  45

Leu Ile Asp Ile Gln Glu Phe Tyr Glu Val Thr Leu Leu Asp Asn Pro  
                   50                                  55                                  60

Lys Cys Ile Asp Arg Ser Lys Pro Ser Glu Pro Ile Gln Pro Val Asn  
                   65                                  70                                  75                                  80

Thr Trp Glu Ile Ser Ser Leu Pro Ser Ser Thr Val Thr Ser Glu Thr  
                                   85                                  90                                  95

Leu Pro Ser Ser Leu Ser Pro Ser Val Glu Lys Tyr Arg Tyr Gln Asp  
                                   100                                  105                                  110

Glu Asp Thr Pro Pro Gln Glu His Ile Ser Pro Gln Ile Thr Asn Glu  
                   115                                  120                                  125

Val Ile Gly Pro Glu Leu Val His Val Ser Glu Lys Asn Leu Ser Glu  
                   130                                  135                                  140

Ile Glu Asn Val His Gly Phe Val Ser His Ser His Ile Ser Pro Ile  
                   145                                  150                                  155                                  160

Lys Pro Thr Glu Ala Val Leu Pro Ser Pro Pro Thr Val Pro Val Ile  
                                   165                                  170                                  175

Pro Val Leu Pro Val Pro Ala Glu Asn Thr Val Ile Leu Pro Thr Ile  
                                   180                                  185                                  190

Pro Gln Ala Asn Pro Pro Pro Val Leu Val Asn Thr Asp Ser Leu Glu  
                   195                                  200                                  205

Thr Pro Thr Tyr Val Asn Gly Thr Asp Ala Asp Tyr Glu Tyr Glu Glu  
                   210                                  215                                  220

Ile Thr Leu Glu Arg Gly Asn Ser Gly Leu Gly Phe Ser Ile Ala Gly  
                   225                                  230                                  235                                  240

Gly Thr Asp Asn Pro His Ile Gly Asp Asp Ser Ser Ile Phe Ile Thr  
                                   245                                  250                                  255

Lys Ile Ile Thr Gly Gly Ala Ala Ala Gln Asp Gly Arg Leu Arg Val

260										265										270																																			
Asn	Asp	Cys	Ile	Leu	Gln	Val	Asn	Glu	Val	Asp	Val	Arg	Asp	Val	Thr																																								
		275								280					285																																								
His	Ser	Lys	Ala	Val	Glu	Ala	Leu	Lys	Glu	Ala	Gly	Ser	Ile	Val	Arg																																								
		290					295				300																																												
Leu	Tyr	Val	Lys	Arg	Arg	Lys	Pro	Val	Ser	Glu	Lys	Ile	Met	Glu	Ile																																								
		305				310					315				320																																								
Lys	Leu	Ile	Lys	Gly	Pro	Lys	Gly	Leu	Gly	Phe	Ser	Ile	Ala	Gly	Gly																																								
				325						330				335																																									
Val	Gly	Asn	Gln	His	Ile	Pro	Gly	Asp	Asn	Ser	Ile	Tyr	Val	Thr	Lys																																								
				340					345					350																																									
Ile	Ile	Glu	Gly	Gly	Ala	Ala	His	Lys	Asp	Gly	Lys	Leu	Gln	Ile	Gly																																								
				355				360					365																																										
Asp	Lys	Leu	Leu	Ala	Val	Asn	Asn	Val	Cys	Leu	Glu	Glu	Val	Thr	His																																								
				370				375				380																																											
Glu	Glu	Ala	Val	Thr	Ala	Leu	Lys	Asn	Thr	Ser	Asp	Phe	Val	Tyr	Leu																																								
				385			390				395				400																																								
Lys	Val	Ala	Lys	Pro	Thr	Ser	Met	Tyr	Met	Asn	Asp	Gly	Tyr	Ala	Pro																																								
				405					410					415																																									
Pro	Asp	Ile	Thr	Asn	Ser	Ser	Ser	Gln	Pro	Val	Asp	Asn	His	Val	Ser																																								
				420				425					430																																										
Pro	Ser	Ser	Phe	Leu	Gly	Gln	Thr	Pro	Ala	Ser	Pro	Ala	Arg	Tyr	Ser																																								
				435				440					445																																										
Pro	Val	Ser	Lys	Ala	Val	Leu	Gly	Asp	Asp	Glu	Ile	Thr	Arg	Glu	Pro																																								
				450			455					460																																											
Arg	Lys	Val	Val	Leu	His	Arg	Gly	Ser	Thr	Gly	Leu	Gly	Phe	Asn	Ile																																								
				465			470				475				480																																								
Val	Gly	Gly	Glu	Asp	Gly	Glu	Gly	Ile	Phe	Ile	Ser	Phe	Ile	Leu	Ala																																								
				485				490						495																																									
Gly	Gly	Pro	Ala	Asp	Leu	Ser	Gly	Glu	Leu	Arg	Lys	Gly	Asp	Arg	Ile																																								
				500				505					510																																										



Ile Ser Val Asn Ser Val Asp Leu Arg Ala Ala Ser His Glu Gln Ala  
 515 520 525  
 Ala Ala Ala Leu Lys Asn Ala Gly Gln Ala Val Thr Ile Val Ala Gln  
 530 535 540  
 Tyr Arg Pro Glu Glu Tyr Ser Arg Phe Glu Ala Lys Ile His Asp Leu  
 545 550 555 560  
 Arg Glu Gln Met Met Asn Ser Ser Ile Ser Ser Gly Ser Gly Ser Leu  
 565 570 575  
 Arg Thr Ser Gln Lys Arg Ser Leu Tyr Val Arg Ala Leu Phe Asp Tyr  
 580 585 590  
 Asp Lys Thr Lys Asp Ser Gly Leu Pro Ser Gln Gly Leu Asn Phe Lys  
 595 600 605  
 Phe Gly Asp Ile Leu His Val Ile Asn Ala Ser Asp Asp Glu Trp Trp  
 610 615 620  
 Gln Ala Arg Gln Val Thr Pro Asp Gly Glu Ser Asp Glu Val Gly Val  
 625 630 635 640  
 Ile Pro Ser Lys Arg Arg Val Glu Lys Lys Glu Arg Ala Arg Leu Lys  
 645 650 655  
 Thr Val Lys Phe Asn Ser Lys Thr Arg Asp Lys Gly Gln Ser Phe Asn  
 660 665 670  
 Asp Lys Arg Lys Lys Asn Leu Phe Ser Arg Lys Phe Pro Phe Tyr Lys  
 675 680 685  
 Asn Lys Asp Gln Ser Glu Gln Glu Thr Ser Asp Ala Asp Gln His Val  
 690 695 700  
 Thr Ser Asn Ala Ser Asp Ser Glu Ser Ser Tyr Arg Gly Gln Glu Glu  
 705 710 715 720  
 Tyr Val Leu Ser Tyr Glu Pro Val Asn Gln Gln Glu Val Asn Tyr Thr  
 725 730 735  
 Arg Pro Val Ile Ile Leu Gly Pro Met Lys Asp Arg Ile Asn Asp Asp  
 740 745 750  
 Leu Ile Ser Glu Phe Pro Asp Lys Phe Gly Ser Cys Val Pro His Thr

755	760	765
Thr Arg Pro Lys Arg Asp Tyr Glu Val Asp Gly Arg Asp Tyr His Phe 770 775 780		
Val Thr Ser Arg Glu Gln Met Glu Lys Asp Ile Gln Glu His Lys Phe 785 790 795 800		
Ile Glu Ala Gly Gln Tyr Asn Asn His Leu Tyr Gly Thr Ser Val Gln 805 810 815		
Ser Val Arg Glu Val Ala Gly Lys Gly Lys His Cys Ile Leu Asp Val 820 825 830		
Ser Gly Asn Ala Ile Lys Arg Leu Gln Ile Ala Gln Leu Tyr Pro Ile 835 840 845		
Ser Ile Phe Ile Lys Pro Lys Ser Met Glu Asn Ile Met Glu Met Asn 850 855 860		
Lys Arg Leu Thr Glu Glu Gln Ala Arg Lys Thr Phe Glu Arg Ala Met 865 870 875 880		
Lys Leu Glu Gln Glu Phe Thr Glu His Phe Thr Ala Ile Val Gln Gly 885 890 895		
Asp Thr Leu Glu Asp Ile Tyr Asn Gln Val Lys Gln Ile Ile Glu Glu 900 905 910		
Gln Ser Gly Ser Tyr Ile Trp Val Pro Ala Lys Glu Lys Leu 915 920 925		
<210> 11		
<211> 870		
<212> PRT		
<213> Homo sapiens		
<400> 11		
Met Phe Phe Ala Cys Tyr Cys Ala Leu Arg Thr Asn Val Lys Lys Tyr 1 5 10 15		
Arg Tyr Gln Asp Glu Asp Ala Pro His Asp His Ser Leu Pro Arg Leu 20 25 30		
Thr His Glu Val Arg Gly Pro Glu Leu Val His Val Ser Glu Lys Asn 35 40 45		

Leu Ser Gln Ile Glu Asn Val His Gly Tyr Val Leu Gln Ser His Ile  
 50 55 60

Ser Pro Leu Lys Ala Ser Pro Ala Pro Ile Ile Val Asn Thr Asp Thr  
 65 70 75 80

Leu Asp Thr Ile Pro Tyr Val Asn Gly Thr Glu Ile Glu Tyr Glu Phe  
 85 90 95

Glu Glu Ile Thr Leu Glu Arg Gly Asn Ser Gly Leu Gly Phe Ser Ile  
 100 105 110

Ala Gly Gly Thr Asp Asn Pro His Ile Gly Asp Asp Pro Gly Ile Phe  
 115 120 125

Ile Thr Lys Ile Ile Pro Gly Gly Ala Ala Ala Glu Asp Gly Arg Leu  
 130 135 140

Arg Val Asn Asp Cys Ile Leu Arg Val Asn Glu Val Asp Val Ser Glu  
 145 150 155 160

Val Ser His Ser Lys Ala Val Glu Ala Leu Lys Glu Ala Gly Ser Ile  
 165 170 175

Ala Arg Leu Tyr Val Arg Arg Arg Arg Pro Ile Leu Glu Thr Val Val  
 180 185 190

Glu Ile Lys Leu Phe Lys Gly Pro Lys Gly Leu Gly Phe Ser Ile Ala  
 195 200 205

Gly Gly Val Gly Asn Gln His Ile Pro Gly Asp Asn Ser Ile Tyr Val  
 210 215 220

Thr Lys Ile Ile Asp Gly Gly Ala Ala Gln Lys Asp Gly Arg Leu Gln  
 225 230 235 240

Val Gly Asp Arg Leu Leu Met Val Asn Asn Tyr Ser Leu Glu Glu Val  
 245 250 255

Thr His Glu Glu Ala Val Ala Ile Leu Lys Asn Thr Ser Glu Val Val  
 260 265 270

Tyr Leu Lys Val Gly Asn Pro Thr Thr Ile Tyr Met Thr Asp Pro Tyr  
 275 280 285

Gly Pro Pro Asp Ile Thr His Ser Tyr Ser Pro Pro Met Glu Asn His  
 290 295 300

Leu Leu Ser Gly Asn Asn Gly Thr Leu Glu Tyr Lys Thr Ser Leu Pro  
 305 310 315 320  
 Pro Ile Ser Pro Gly Arg Tyr Ser Pro Ile Pro Lys His Met Leu Val  
 325 330 335  
 Asp Asp Asp Tyr Thr Arg Pro Pro Glu Pro Val Tyr Ser Thr Val Asn  
 340 345 350  
 Lys Leu Cys Asp Lys Pro Ala Ser Pro Arg His Tyr Ser Pro Val Glu  
 355 360 365  
 Cys Asp Lys Ser Phe Leu Leu Ser Ala Pro Tyr Ser His Tyr His Leu  
 370 375 380  
 Gly Leu Leu Pro Asp Ser Glu Met Thr Ser His Ser Gln His Ser Thr  
 385 390 395 400  
 Ala Thr Arg Gln Pro Ser Met Thr Leu Gln Arg Ala Val Ser Leu Glu  
 405 410 415  
 Gly Glu Pro Arg Lys Val Val Leu His Lys Gly Ser Thr Gly Leu Gly  
 420 425 430  
 Phe Asn Ile Val Gly Gly Glu Asp Gly Glu Gly Ile Phe Val Ser Phe  
 435 440 445  
 Ile Leu Ala Gly Gly Pro Ala Asp Leu Ser Gly Glu Leu Gln Arg Gly  
 450 455 460  
 Asp Gln Ile Leu Ser Val Asn Gly Ile Asp Leu Arg Gly Ala Ser His  
 465 470 475 480  
 Glu Gln Ala Ala Ala Ala Leu Lys Gly Ala Gly Gln Thr Val Thr Ile  
 485 490 495  
 Ile Ala Gln Tyr Gln Pro Glu Asp Tyr Ala Arg Phe Glu Ala Lys Ile  
 500 505 510  
 His Asp Leu Arg Glu Gln Met Met Asn His Ser Met Ser Ser Gly Ser  
 515 520 525  
 Gly Ser Leu Arg Thr Asn Gln Lys Arg Ser Leu Tyr Val Arg Ala Met  
 530 535 540

Phe Asp Tyr Asp Lys Ser Lys Asp Ser Gly Leu Pro Ser Gln Gly Leu  
 545 550 555 560

Ser Phe Lys Tyr Gly Asp Ile Leu His Val Ile Asn Ala Ser Asp Asp  
 565 570 575

Glu Trp Trp Gln Ala Arg Arg Val Met Leu Glu Gly Asp Ser Glu Glu  
 580 585 590

Met Gly Val Ile Pro Ser Lys Arg Arg Val Glu Arg Lys Glu Arg Ala  
 595 600 605

Arg Leu Lys Thr Val Lys Phe Asn Ala Lys Pro Gly Val Ile Asp Ser  
 610 615 620

Lys Gly Ser Phe Asn Asp Lys Arg Lys Lys Ser Phe Ile Phe Ser Arg  
 625 630 635 640

Lys Phe Pro Phe Tyr Lys Asn Lys Glu Gln Ser Glu Gln Glu Thr Ser  
 645 650 655

Asp Pro Glu Arg Gly Gln Glu Asp Leu Ile Leu Ser Tyr Glu Pro Val  
 660 665 670

Thr Arg Gln Glu Ile Asn Tyr Thr Arg Pro Val Ile Ile Leu Gly Pro  
 675 680 685

Met Lys Asp Arg Ile Asn Asp Asp Leu Ile Ser Glu Phe Pro Asp Lys  
 690 695 700

Phe Gly Ser Cys Val Pro His Thr Thr Arg Pro Lys Arg Asp Tyr Glu  
 705 710 715 720

Val Asp Gly Arg Asp Tyr His Phe Val Ile Ser Arg Glu Gln Met Glu  
 725 730 735

Lys Asp Ile Gln Glu His Lys Phe Ile Glu Ala Gly Gln Tyr Asn Asp  
 740 745 750

Asn Leu Tyr Gly Thr Ser Val Gln Ser Val Arg Phe Val Ala Glu Arg  
 755 760 765

Gly Lys His Cys Ile Leu Asp Val Ser Gly Asn Ala Ile Lys Arg Leu  
 770 775 780

Gln Val Ala Gln Leu Tyr Pro Ile Ala Ile Phe Ile Lys Pro Arg Ser  
 785 790 795 800

Leu Glu Ser Leu Met Glu Met Asn Lys Arg Leu Thr Glu Glu Gln Ala  
805 810 815

Lys Lys Thr Tyr Asp Arg Ala Ile Lys Leu Glu Gln Glu Phe Gly Glu  
820 825 830

Tyr Phe Thr Ala Ile Val Gln Gly Asp Thr Leu Glu Asp Ile Tyr Asn  
835 840 845

Gln Cys Lys Leu Val Ile Glu Glu Gln Ser Gly Pro Phe Ile Trp Ile  
850 855 860

Pro Ser Lys Glu Lys Leu  
865 870

<210> 12  
<211> 817  
<212> PRT  
<213> Homo sapiens

<400> 12

Met His Lys His Gln His Cys Cys Lys Cys Pro Glu Cys Tyr Glu Val  
1 5 10 15

Thr Arg Leu Ala Ala Leu Arg Arg Leu Glu Pro Pro Gly Tyr Gly Asp  
20 25 30

Trp Gln Val Pro Asp Pro Tyr Gly Pro Gly Gly Gly Asn Gly Ala Ser  
35 40 45

Ala Gly Tyr Gly Gly Tyr Ser Ser Gln Thr Leu Pro Ser Gln Ala Gly  
50 55 60

Ala Thr Pro Thr Pro Arg Thr Lys Ala Lys Leu Ile Pro Thr Gly Arg  
65 70 75 80

Asp Val Gly Pro Val Pro Leu Lys Pro Val Pro Gly Lys Ser Thr Pro  
85 90 95

Lys Leu Asn Gly Ser Gly Pro Ser Trp Trp Pro Glu Cys Thr Cys Thr  
100 105 110

Asn Arg Asp Trp Tyr Glu Gln Val Asn Gly Ser Asp Gly Met Phe Lys  
115 120 125

Tyr Glu Glu Ile Val Leu Glu Arg Gly Asn Ser Gly Leu Gly Phe Ser

130	135	140
Ile Ala Gly Gly Ile Asp Asn Pro His Val Pro Asp Asp Pro Gly Ile		
145	150	155 160
Phe Ile Thr Lys Ile Ile Pro Gly Gly Ala Ala Ala Met Asp Gly Arg		
	165	170 175
Leu Gly Val Asn Asp Cys Val Leu Arg Val Asn Glu Val Glu Val Ser		
	180	185 190
Glu Val Val His Ser Arg Ala Val Glu Ala Leu Lys Glu Ala Gly Pro		
	195	200 205
Val Val Arg Leu Val Val Arg Arg Arg Gln Pro Pro Pro Glu Thr Ile		
	210	215 220
Met Glu Val Asn Leu Leu Lys Gly Pro Lys Gly Leu Gly Phe Ser Ile		
	225	230 235 240
Ala Gly Gly Ile Gly Asn Gln His Ile Pro Gly Asp Asn Ser Ile Tyr		
	245	250 255
Ile Thr Lys Ile Ile Glu Gly Gly Ala Ala Gln Lys Asp Gly Arg Leu		
	260	265 270
Gln Ile Gly Asp Arg Leu Leu Ala Val Asn Asn Thr Asn Leu Gln Asp		
	275	280 285
Val Arg His Glu Glu Ala Val Ala Ser Leu Lys Asn Thr Ser Asp Met		
	290	295 300
Val Tyr Leu Lys Val Ala Lys Pro Gly Ser Leu His Leu Asn Asp Met		
	305	310 315 320
Tyr Ala Pro Pro Asp Tyr Ala Ser Thr Phe Thr Ala Leu Ala Asp Asn		
	325	330 335
His Ile Ser His Asn Ser Ser Leu Gly Tyr Leu Gly Ala Val Glu Ser		
	340	345 350
Lys Val Ser Tyr Pro Ala Pro Pro Gln Val Pro Pro Thr Arg Tyr Ser		
	355	360 365
Pro Ile Pro Arg His Met Leu Ala Glu Glu Asp Phe Thr Arg Glu Pro		
	370	375 380

Arg Lys Ile Ile Leu His Lys Gly Ser Thr Gly Leu Gly Phe Asn Ile  
 385 390 395 400  
 Val Gly Gly Glu Asp Gly Glu Gly Ile Phe Val Ser Phe Ile Leu Ala  
 405 410 415  
 Gly Gly Pro Ala Asp Leu Ser Gly Glu Leu Arg Arg Gly Asp Arg Ile  
 420 425 430  
 Leu Ser Val Asn Gly Val Asn Leu Arg Asn Ala Thr His Glu Gln Ala  
 435 440 445  
 Ala Ala Ala Leu Lys Arg Ala Gly Gln Ser Val Thr Ile Val Ala Gln  
 450 455 460  
 Tyr Arg Pro Glu Glu Tyr Ser Arg Phe Glu Ser Lys Ile His Asp Leu  
 465 470 475 480  
 Arg Glu Gln Met Met Asn Ser Ser Met Ser Ser Gly Ser Gly Ser Leu  
 485 490 495  
 Arg Thr Ser Glu Lys Arg Ser Leu Tyr Val Arg Ala Leu Phe Asp Tyr  
 500 505 510  
 Asp Arg Thr Arg Asp Ser Cys Leu Pro Ser Gln Gly Leu Ser Phe Ser  
 515 520 525  
 Tyr Gly Asp Ile Leu His Val Ile Asn Ala Ser Asp Asp Glu Trp Trp  
 530 535 540  
 Gln Ala Arg Leu Val Thr Pro His Gly Glu Ser Glu Gln Ile Gly Val  
 545 550 555 560  
 Ile Pro Ser Lys Lys Arg Val Glu Lys Lys Glu Arg Ala Arg Leu Lys  
 565 570 575  
 Thr Val Lys Phe His Ala Arg Thr Gly Met Ile Glu Ser Asn Arg Asp  
 580 585 590  
 Phe Pro Gly Leu Ser Asp Asp Tyr Tyr Gly Ala Lys Asn Leu Lys Gly  
 595 600 605  
 Gln Glu Asp Ala Ile Leu Ser Tyr Glu Pro Val Thr Arg Gln Glu Ile  
 610 615 620  
 His Tyr Ala Arg Pro Val Ile Ile Leu Gly Pro Met Lys Asp Arg Val



625                                      630                                      635                                      640  
 Asn Asp Asp Leu Ile Ser Glu Phe Pro His Lys Phe Gly Ser Cys Val  
    645                                      650                                      655  
 Pro His Thr Thr Arg Pro Arg Arg Asp Asn Glu Val Asp Gly Gln Asp  
    660                                      665                                      670  
 Tyr His Phe Val Val Ser Arg Glu Gln Met Glu Lys Asp Ile Gln Asp  
    675                                      680                                      685  
 Asn Lys Phe Ile Glu Ala Gly Gln Phe Asn Asp Asn Leu Tyr Gly Thr  
    690                                      695                                      700  
 Ser Ile Gln Ser Val Arg Ala Val Ala Glu Arg Gly Lys His Cys Ile  
    705                                      710                                      715                                      720  
 Leu Asp Val Ser Gly Asn Ala Ile Lys Arg Leu Gln Gln Ala Gln Leu  
    725                                      730                                      735  
 Tyr Pro Ile Ala Ile Phe Ile Lys Pro Lys Ser Ile Glu Ala Leu Met  
    740                                      745                                      750  
 Glu Met Asn Arg Arg Gln Thr Tyr Glu Gln Ala Asn Lys Ile Tyr Asp  
    755                                      760                                      765  
 Lys Ala Met Lys Leu Glu Gln Glu Phe Gly Glu Tyr Phe Thr Ala Ile  
    770                                      775                                      780  
 Val Gln Gly Asp Ser Leu Glu Glu Ile Tyr Asn Lys Ile Lys Gln Ile  
    785                                      790                                      795                                      800  
 Ile Glu Asp Gln Ser Gly His Tyr Ile Trp Val Pro Ser Pro Glu Lys  
    805                                      810                                      815  
 Leu  
  
 <210> 13  
 <211> 767  
 <212> PRT  
 <213> Homo sapiens  
  
 <400> 13  
 Met Ser Gln Arg Pro Arg Ala Pro Arg Ser Ala Leu Trp Leu Leu Ala  
 1                                      5                                      10                                      15

Pro Pro Leu Leu Arg Trp Ala Pro Pro Leu Leu Thr Val Leu His Ser  
 20 25 30

Asp Leu Phe Gln Ala Leu Leu Asp Ile Leu Asp Tyr Tyr Glu Ala Ser  
 35 40 45

Leu Ser Glu Ser Gln Lys Tyr Arg Tyr Gln Asp Glu Asp Thr Pro Pro  
 50 55 60

Leu Glu His Ser Pro Ala His Leu Pro Asn Gln Ala Asn Ser Pro Pro  
 65 70 75 80

Val Ile Val Asn Thr Asp Thr Leu Glu Ala Pro Gly Tyr Glu Leu Gln  
 85 90 95

Val Asn Gly Thr Glu Gly Glu Met Glu Tyr Glu Glu Ile Thr Leu Glu  
 100 105 110

Arg Gly Asn Ser Gly Leu Gly Phe Ser Ile Ala Gly Gly Thr Asp Asn  
 115 120 125

Pro His Ile Gly Asp Asp Pro Ser Ile Phe Ile Thr Lys Ile Ile Pro  
 130 135 140

Gly Gly Ala Ala Ala Gln Asp Gly Arg Leu Arg Val Asn Asp Ser Ile  
 145 150 155 160

Leu Phe Val Asn Glu Val Asp Val Arg Glu Val Thr His Ser Ala Ala  
 165 170 175

Val Glu Ala Leu Lys Glu Ala Gly Ser Ile Val Arg Leu Tyr Val Met  
 180 185 190

Arg Arg Lys Pro Pro Ala Glu Lys Val Met Glu Ile Lys Leu Ile Lys  
 195 200 205

Gly Pro Lys Gly Leu Gly Phe Ser Ile Ala Gly Gly Val Gly Asn Gln  
 210 215 220

His Ile Pro Gly Asp Asn Ser Ile Tyr Val Thr Lys Ile Ile Glu Gly  
 225 230 235 240

Gly Ala Ala His Lys Asp Gly Arg Leu Gln Ile Gly Asp Lys Ile Leu  
 245 250 255

Ala Val Asn Ser Val Gly Leu Glu Asp Val Met His Glu Asp Ala Val  
 260 265 270

Ala Ala Leu Lys Asn Thr Tyr Asp Val Val Tyr Leu Lys Val Ala Lys  
 275 280 285

Pro Ser Asn Ala Tyr Leu Ser Asp Ser Tyr Ala Pro Pro Asp Ile Thr  
 290 295 300

Thr Ser Tyr Ser Gln His Leu Asp Asn Glu Ile Ser His Ser Ser Tyr  
 305 310 315 320

Leu Gly Thr Asp Tyr Pro Thr Ala Met Thr Pro Thr Ser Pro Arg Arg  
 325 330 335

Tyr Ser Pro Val Ala Lys Asp Leu Leu Gly Glu Glu Asp Ile Pro Arg  
 340 345 350

Glu Pro Arg Arg Ile Val Ile His Arg Gly Ser Thr Gly Leu Gly Phe  
 355 360 365

Asn Ile Val Gly Gly Glu Asp Gly Glu Gly Ile Phe Ile Ser Phe Ile  
 370 375 380

Leu Ala Gly Gly Pro Ala Asp Leu Ser Gly Glu Leu Arg Lys Gly Asp  
 385 390 395 400

Gln Ile Leu Ser Val Asn Gly Val Asp Leu Arg Asn Ala Ser His Glu  
 405 410 415

Gln Ala Ala Ile Ala Leu Lys Asn Ala Gly Gln Thr Val Thr Ile Ile  
 420 425 430

Ala Gln Tyr Lys Pro Glu Glu Tyr Ser Arg Phe Glu Ala Lys Ile His  
 435 440 445

Asp Leu Arg Glu Gln Leu Met Asn Ser Ser Leu Gly Ser Gly Thr Ala  
 450 455 460

Ser Leu Arg Ser Asn Pro Lys Arg Gly Phe Tyr Ile Arg Ala Leu Phe  
 465 470 475 480

Asp Tyr Asp Lys Thr Lys Asp Cys Gly Phe Leu Ser Gln Ala Leu Ser  
 485 490 495

Phe Arg Phe Gly Asp Val Leu His Val Ile Asp Ala Ser Asp Glu Glu  
 500 505 510

Trp Trp Gln Ala Arg Arg Val His Ser Asp Ser Glu Thr Asp Asp Ile  
 515 520 525

Gly Phe Ile Pro Ser Lys Arg Arg Val Glu Arg Arg Glu Trp Ser Arg  
 530 535 540

Leu Lys Ala Lys Asp Trp Gly Ser Ser Ser Gly Ser Gln Gly Arg Glu  
 545 550 555 560

Asp Ser Val Leu Ser Tyr Glu Thr Val Thr Gln Met Glu Val His Tyr  
 565 570 575

Ala Arg Pro Ile Ile Ile Leu Gly Pro Thr Lys Asp Arg Ala Asn Asp  
 580 585 590

Asp Leu Leu Ser Glu Phe Pro Asp Lys Phe Gly Ser Cys Val Pro His  
 595 600 605

Thr Thr Arg Pro Lys Arg Glu Tyr Glu Ile Asp Gly Arg Asp Tyr His  
 610 615 620

Phe Val Ser Ser Arg Glu Lys Met Glu Lys Asp Ile Gln Ala His Lys  
 625 630 635 640

Phe Ile Glu Ala Gly Gln Tyr Asn Ser His Leu Tyr Gly Thr Ser Val  
 645 650 655

Gln Ser Val Arg Glu Val Ala Glu Gln Gly Lys His Cys Ile Leu Asp  
 660 665 670

Val Ser Ala Asn Ala Val Arg Arg Leu Gln Ala Ala His Leu His Pro  
 675 680 685

Ile Ala Ile Phe Ile Arg Pro Arg Ser Leu Glu Asn Val Leu Glu Ile  
 690 695 700

Asn Lys Arg Ile Thr Glu Glu Gln Ala Arg Lys Ala Phe Asp Arg Ala  
 705 710 715 720

Thr Lys Leu Glu Gln Glu Phe Thr Glu Cys Phe Ser Ala Ile Val Glu  
 725 730 735

Gly Asp Ser Phe Glu Glu Ile Tyr His Lys Val Lys Arg Val Ile Glu  
 740 745 750

Asp Leu Ser Gly Pro Tyr Ile Trp Val Pro Ala Arg Glu Arg Leu  
 755 760 765

<210> 14  
 <211> 512  
 <212> PRT  
 <213> Homo sapiens

<400> 14

Met Glu Arg Ala Arg Lys Phe Ser Gly Ser Gly Leu Ala Met Gly Leu  
 1 5 10 15

Gly Ser Ala Ser Ala Ser Ala Trp Arg Arg Ala Ser Gln Arg Trp Ala  
 20 25 30

Trp Pro Leu Arg Ser Leu Arg Pro Gly Gly Asp Ala Arg Glu Pro Arg  
 35 40 45

Lys Ile Ile Leu His Lys Gly Ser Thr Gly Leu Gly Phe Asn Ile Val  
 50 55 60

Gly Gly Glu Asp Gly Glu Gly Ile Phe Val Ser Phe Ile Leu Ala Gly  
 65 70 75 80

Gly Pro Ala Asp Leu Ser Gly Glu Leu Arg Arg Gly Asp Arg Ile Leu  
 85 90 95

Ser Val Asn Gly Val Asn Leu Arg Asn Ala Thr His Glu Gln Ala Ala  
 100 105 110

Ala Ala Leu Lys Arg Ala Gly Gln Ser Val Thr Ile Val Ala Gln Tyr  
 115 120 125

Arg Pro Glu Glu Tyr Ser Arg Phe Glu Ser Lys Ile His Asp Leu Arg  
 130 135 140

Glu Gln Met Met Asn Ser Ser Met Ser Ser Gly Ser Gly Ser Leu Arg  
 145 150 155 160

Thr Ser Glu Lys Arg Ser Leu Tyr Val Arg Ala Leu Phe Asp Tyr Asp  
 165 170 175

Arg Thr Arg Asp Ser Cys Leu Pro Ser Gln Gly Leu Ser Phe Ser Tyr  
 180 185 190

Gly Asp Ile Leu His Val Ile Asn Ala Ser Asp Asp Glu Trp Trp Gln  
 195 200 205

Ala Arg Leu Val Thr Pro His Gly Glu Ser Glu Gln Ile Gly Val Ile

210	215	220
Pro Ser Lys Lys Arg Val Glu Lys Lys Glu Arg Ala Arg Leu Lys Thr 225 230 235 240		
Val Lys Phe His Ala Arg Thr Gly Met Ile Glu Ser Asn Arg Ser Ile 245 250 255		
Lys Thr Lys Arg Lys Lys Ser Phe Arg Leu Ser Arg Lys Phe Pro Phe 260 265 270		
Tyr Lys Ser Lys Glu Asn Met Ala Gln Glu Ser Ser Ile Gln Glu Gln 275 280 285		
Gly Val Thr Ser Asn Thr Ser Asp Ser Glu Ser Ser Ser Lys Gly Gln 290 295 300		
Glu Asp Ala Ile Leu Ser Tyr Glu Pro Val Thr Arg Gln Glu Ile His 305 310 315 320		
Tyr Ala Arg Pro Val Ile Ile Leu Gly Pro Met Lys Asp Arg Val Asn 325 330 335		
Asp Asp Leu Ile Ser Glu Phe Pro His Lys Phe Gly Ser Cys Val Pro 340 345 350		
His Thr Thr Arg Pro Arg Arg Asp Asn Glu Val Asp Gly Gln Asp Tyr 355 360 365		
His Phe Val Val Ser Arg Glu Gln Met Glu Lys Asp Ile Gln Asp Asn 370 375 380		
Lys Phe Ile Glu Ala Gly Gln Phe Asn Asp Asn Leu Tyr Gly Thr Ser 385 390 395 400		
Ile Gln Ser Val Arg Ala Val Ala Glu Arg Gly Lys His Cys Ile Leu 405 410 415		
Asp Val Ser Gly Asn Ala Ile Lys Arg Leu Gln Gln Ala Gln Leu Tyr 420 425 430		
Pro Ile Ala Ile Phe Ile Lys Pro Lys Ser Ile Glu Ala Leu Met Glu 435 440 445		
Met Asn Arg Arg Gln Thr Tyr Glu Gln Ala Asn Lys Ile Tyr Asp Lys 450 455 460		

Ala Met Lys Leu Glu Gln Glu Phe Gly Glu Tyr Phe Thr Ala Ile Val  
 465 470 475 480

Gln Gly Asp Ser Leu Glu Glu Ile Tyr Asn Lys Ile Lys Gln Ile Ile  
 485 490 495

Glu Asp Gln Ser Gly His Tyr Ile Trp Val Pro Ser Pro Glu Lys Leu  
 500 505 510

<210> 15  
 <211> 178  
 <212> PRT  
 <213> Homo sapiens

<400> 15

Met Pro Lys Ala Lys Gly Lys Thr Arg Arg Gln Lys Phe Gly Tyr Ser  
 1 5 10 15

Val Asn Arg Lys Arg Leu Asn Arg Asn Ala Arg Arg Lys Ala Ala Arg  
 20 25 30

Gly Ile Glu Cys Ser His Ile Arg His Ala Trp Asp His Ala Lys Ser  
 35 40 45

Val Arg Gln Asn Leu Ala Glu Met Gly Leu Ala Val Asp Pro Asn Arg  
 50 55 60

Ala Val Pro Leu Arg Lys Arg Lys Val Lys Ala Met Glu Val Asp Ile  
 65 70 75 80

Glu Glu Arg Pro Lys Glu Leu Val Arg Lys Pro Tyr Val Leu Asn Asp  
 85 90 95

Leu Glu Ala Glu Ala Ser Leu Pro Glu Lys Lys Gly Asn Thr Leu Ser  
 100 105 110

Arg Asp Leu Ile Asp Tyr Val Arg Tyr Met Val Glu Asn His Gly Glu  
 115 120 125

Asp Tyr Lys Ala Met Ala Arg Asp Glu Lys Asn Tyr Tyr Gln Asp Thr  
 130 135 140

Pro Lys Gln Ile Arg Ser Lys Ile Asn Val Tyr Lys Arg Phe Tyr Pro  
 145 150 155 160

Ala Glu Trp Gln Asp Phe Leu Asp Ser Leu Gln Lys Arg Lys Met Glu  
 165 170 175

Val Glu

<210> 16  
 <211> 221  
 <212> PRT  
 <213> Homo sapiens

&lt;400&gt; 16

Met Glu Ala Gly Gly Phe Leu Asp Ser Leu Ile Tyr Gly Ala Cys Val  
 1 5 10 15

Val Phe Thr Leu Gly Met Phe Ser Ala Gly Leu Ser Asp Leu Arg His  
 20 25 30

Met Arg Met Thr Arg Ser Val Asp Asn Val Arg Phe Leu Pro Phe Leu  
 35 40 45

Thr Thr Glu Val Asn Asn Leu Gly Trp Leu Ser Tyr Gly Ala Leu Lys  
 50 55 60

Gly Asp Gly Ile Leu Ile Val Val Asn Thr Val Gly Ala Ala Leu Gln  
 65 70 75 80

Thr Leu Tyr Ile Leu Ala Tyr Leu His Tyr Cys Pro Arg Lys Arg Val  
 85 90 95

Val Leu Leu Gln Thr Ala Thr Leu Leu Gly Val Leu Leu Leu Gly Tyr  
 100 105 110

Gly Tyr Phe Trp Leu Leu Val Pro Asn Pro Glu Ala Arg Leu Gln Gln  
 115 120 125

Leu Gly Leu Phe Cys Ser Val Phe Thr Ile Ser Met Tyr Leu Ser Pro  
 130 135 140

Leu Ala Asp Leu Ala Lys Val Ile Gln Thr Lys Ser Thr Gln Cys Leu  
 145 150 155 160

Ser Tyr Pro Leu Thr Ile Ala Thr Leu Leu Thr Ser Ala Ser Trp Cys  
 165 170 175

Leu Tyr Gly Phe Arg Leu Arg Asp Pro Tyr Ile Met Val Ser Asn Phe  
 180 185 190

Pro Gly Ile Val Thr Ser Phe Ile Arg Phe Trp Leu Phe Trp Lys Tyr



195

200

205

Pro Gln Glu Gln Asp Arg Asn Tyr Trp Leu Leu Gln Thr  
210 215 220



(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
26 June 2003 (26.06.2003)

PCT

(10) International Publication Number  
**WO 03/052068 A3**

(51) International Patent Classification<sup>7</sup>: **C07H 21/04**,  
C12N 15/00, G01N 33/53

(74) Agents: **SHAYESTEH**, Laleh et al.; Exelixis, Inc., P.O.  
Box 511, 170 Harbor Way, South San Francisco, CA  
94083-0511 (US).

(21) International Application Number: PCT/US02/39796

(22) International Filing Date:  
12 December 2002 (12.12.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/340,213 13 December 2001 (13.12.2001) US  
60/340,314 13 December 2001 (13.12.2001) US  
60/340,322 13 December 2001 (13.12.2001) US  
60/357,502 15 February 2002 (15.02.2002) US

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,  
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,  
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,  
SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN,  
YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),  
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,  
ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK,  
TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,  
GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*): **EX-  
ELIXIS, INC.** [US/US]; P.O. Box 511, 170 Harbor Way,  
South San Francisco, CA 94083-0511 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **COSTA, Michael**,  
A. [US/US]; 18 Hazelwood Avenue, San Francisco, CA  
94112 (US). **GENDREAU, Steven, Brian** [US/US]; 2801  
Turk Street, #3, San Francisco, CA 94118 (US). **DORA,**  
**Emery, G., III** [US/US]; 1847 32nd Avenue, San Fran-  
cisco, CA 94122 (US). **NICOLL, Monique** [US/US]; 224  
Naomi Avenue, Pacifica, CA 94044 (US).

Published:

— with international search report

(88) Date of publication of the international search report:  
9 October 2003

*For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.*

(54) Title: MBCATS AS MODIFIERS OF THE BETA-CATENIN PATHWAY AND METHODS OF USE

(57) Abstract: Human MBCAT genes are identified as modulators of the beta-catenin pathway, and thus are therapeutic targets for disorders associated with defective beta-catenin function. Methods for identifying modulators of beta-catenin, comprising screening for agents that modulate the activity of MBCAT are provided.



WO 03/052068 A3

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/39796

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07H 21/04; C12N 15/00; G01N 33/53

US CL : 536/23.5; 435/325, 7.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 435/325, 7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
CAS ONLINE, APS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,303,576 B1 (BLASCHUK et al) 16 October 2001 (16.10.01) columns 10-14.	1-7, 16-17
Y	US 5,998,600 A (BARKER et al) 07 December 1999 (07.12.99) columns 6-8.	1-7, 16, 17
A	PEER BORK. Powers and Pitfalls in Sequence Analysis: The 70% hurdle. Genome Research. 2000, Vol. 10, pages 398-400, particularly page 398, columns 1 and 2.	1-7, 16, 17

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

09 June 2003 (09.06.2003)

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703)305-3230

Date of mailing of the international search report

30 JUN 2003

Authorized officer

Dave T. Nguyen

Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/39796

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-7, 16, 17 (in-part)

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

The inventions listed as Group I-XXIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Each group of the listed groups is either directed to a distinct goal or a materially distinct step wherein a distinct patient or a distinct sample is employed for the respective goal. For example, a nucleic acid therapy method is not the same as a small molecular weight drug employed treatment nor is it the same as an antibody treatment method. An *ex vivo* binding assay wherein a distinct molecule (antibody, a DNA) is employed is not the same as an *in vivo* binding assay. Likewise, a method of effecting a phenotypic change in an animal model system is not the same as a simple binding assay nor does it have anything to do with an already identified candidate beta catenin pathway modulating agent as clearly set forth in the preamble of claim 1. Also treatment methods wherein a distinct molecule is employed for targeting a specific MBCAT are distinct with one another. As the result, each method requires a specific technical feature and/or distinct goal. Further, 37 CFR 1.475 does not provide for multiple independent products, methods of manufacture and methods of use (37 CFR 1.475(d)).

The International Search Authority has found 23 inventions claimed in the International Application covered by the claims indicated below:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-7, 16-17, drawn to a binding assay wherein a MBCAT polypeptide is employed in a cultured cell.

Group II, claims 1-7, 16, 18-19, embraces an *in vivo* binding assay wherein a MBCAT polypeptide is employed.

Group III, claims 1-6, 8-10, 16-17, drawn to a binding assay wherein a nucleic acid encoding MBCAT is employed in a cultured cell.

Group IV, claims 1-6, 8-10, 18-19, drawn to an *in vivo* binding assay wherein a nucleic acid encoding MBCAT is employed in an animal that mis-expresses a beta-catenin pathway gene.

Group V, claims 11-12, drawn to a method of employing an agent identified in the assay of Group I to effect a phenotypic change in an animal model.

Group VI, claims 11-12, drawn to a method of employing an agent identified in the assay of Group II to effect a phenotypic change in an animal model.

Group VII, claims 13-15, 20-22, drawn to an antibody treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 10.

Group VIII, claims 13-15, 20-22, drawn to an antibody treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 11.

Group IX, claims 13-15, 20-22, drawn to an antibody treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 12.

Group X, claims 13-15, 20-22, drawn to an antibody treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 13.

Group XI, claims 13-15, 20-22, drawn to an antibody treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 14.

Group XII, claims 13-15, 20-22, function as the result of the presence of SEQ ID NO: 15.

## INTERNATIONAL SEARCH REPORT

PCT/US02/39796

Group XIII, claims 13-15, 20-22, drawn to an antibody treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 15.

Group XIV, claims 13-15, 20-22, drawn to an antibody treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 16.

Group XV, claims 13-15, 20-22, drawn to a small molecular weight drug employed treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 10.

Group XVI, claims 13-15, 20-22, drawn to a small molecular weight drug employed treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 11.

Group XVII, claims 13-15, 20-22, drawn to a small molecular weight drug employed treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 12.

Group XVIII, claims 13-15, 20-22, drawn to a small molecular weight drug employed treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 13.

Group XIX, claims 13-15, 20-22, drawn to a small molecular weight drug employed treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 14.

Group XX, claims 13-15, 20-22, drawn to a small molecular weight drug employed treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 15.

Group XXI, claims 13-15, 20-22, drawn to a small molecular weight drug employed treatment method wherein a cell having a defective beta-catenin function as the result of the presence of SEQ ID NO: 16.

Group XXII, claims 20-22, drawn to a nucleic acid therapy method for treating a beta-catenin pathway associated disorder comprising the use of a nucleic acid modulator.

Group XXIII, claims 23-25, drawn to a diagnostic method for detecting a cancer in a subject.

